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5490

The Absorption Spectrum of Medullated and of Non-medullated Nerves.*

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That the conduction of the nerve impulse depends in part at least upon processes of oxidation and reduction is becoming constantly more apparent. It has already been demonstrated that somewhere in the cycle of reactions which produce the nerve impulse, oxygen activated by Warburg's respiratory enzyme is required (Schmitt[1]). The present investigation was undertaken with the purpose of determining the possible rôle of the intracellular hemin compounds (Keilin[2] and Warburg[3]) in nerve function.

The freshly dissected nerves were placed on a slide or in a vessel especially constructed for the purpose and examined with a Zeiss spectromicroscope. The nerves (usually 4 to 8 for each examination) were piled in a heap in order to provide sufficient thickness of material. The claw nerves of the Homarus and of Limulus served as types of non-medullated nerves while green frog sciatics were used as examples of medullated nerves.

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* The experiments upon non-medullated nerves were performed for the greater part at the Marine Biological Laboratory, Woods Hole, Mass. The expense was defrayed in part by a grant to Washington University by the Rockefeller Foundation.

[1] Schmitt, *Am. J. Physiol.*, 1930, **95**, 650.

[2] Keilin, *Proc. Roy. Soc. Ser. B.*, 1930, **106**, 418.

[3] Warburg, *Biochem. Z.*, 1930, **227**, 245.

It was found that the absorption spectrum of the nerves of Homarus and of Limulus resembled each other closely, each presenting 2 distinct bands. The spectrum consisted of one fairly dark band extending from 552 to 562$\mu\mu$ with its center approximately at 557$\mu\mu$, and a second band which, when observable, was located at 525$\mu\mu$. For the same thickness of material the bands were much more intense in Limulus nerves than in Homarus nerves.

Such a two-banded spectrum is characteristic of hemochromogen compounds and from the position of the bands it might be supposed that they represent one component of cytochrome. This seems unlikely, however, since the bands may be observed in fresh nerves in sea water immediately after removal from the animal without the addition of a reducer. Unfortunately, in order to see the bands at all it is necessary to pile the nerves and it is entirely possible this renders them somewhat anaerobic, in which case one would expect the bands of reduced cytochrome to appear. On the other hand, placing the nerves in pure oxygen or treating them with 5% ethyl urethane, which should poison the reducing mechanism, had no noticeable effect on the spectrum. Nor could any consistent change be observed when the nerves were placed in pure nitrogen or when treated with strong reducing agents. While it cannot definitely be stated the absorption spectrum of non-medullated nerves is not that of cytochrome, it is clear that the hemochromogen present in these nerves does not react to reagents as does cytochrome. In contradistinction to nerve, however, muscles of Limulus, Homarus, or frog clearly show four-banded spectra typical of cytochrome, although the action of reagents is not in all cases typical of cytochrome as described by Keilin.

When freshly dissected sciatic nerves of green frogs are examined in the manner described, 2 faint bands make their appearance, which, upon the addition of strong alkali and a reducer are transformed into the more intense bands (at 561 and 525$\mu\mu$) of globin hemochromogen. It can be demonstrated that these bands are due to hemoglobin in the capillaries supplying the nerve fibers rather than to any compound in the fibers themselves. If for a short time preliminary to dissecting the nerves the frogs are perfused with Ringer solution no bands appear even in the presence of alkali and reducer.

Neither cytochrome nor any hemochromogen having a similar spectrum is present in frog nerve in detectable concentrations. The spectrum differs from that of non-medullated nerves also in that there is very marked general absorption in the blue approximately to 522$\mu\mu$. Treating these nerves with pure nitrogen, with a reducer, or with sodium cyanide in concentrations from N/1000 to N/1 has no effect on the spectrum. Ethyl urethane likewise has no effect.

In an effort to determine the nature of the substances responsible for the general absorption in frog nerve, an ether extract of the dried nerves was made. This extract exhibited the same spectrum as was observed in the intact nerves. An excess of acetone was then added to the ether extract and the solution filtered. The precipitate so obtained, consisting chiefly of the nerve phosphatids, was examined and found to give a spectrum very similar to that of the original extract, though much fainter. The filtrate was evaporated to dryness and examined spectroscopically. This fraction exhibited 2 distinct bands located in the region of 500 and 465$\mu\mu$, respectively. The substance responsible for these bands is doubtless a carotinoid pigment, possibly carotin or xanthopyll. The total absorption extending to about 520$\mu\mu$ observed in the intact nerves is due, therefore, to the 2 wide bands of the carotinoid pigments superimposed upon the general absorption of the phosphatids and allied substances.

Using the method devised by Connor[4] for the quantitative determination of the carotin content of tissues it was estimated that nerve contains approximately 0.5 mg. of carotin per gram dry nerve substance. If a solution of this pigment is allowed to stand for any length of time it loses its yellow color and the characteristic absorption bands. This bleaching is due to the spontaneous absorption of oxygen by the carotin (Willstätter and Escher[5]).

Arnaud[6] suggested that carotin may function in plants in a manner similar to that in which hemoglobin functions in blood. The possibility that it may act as an oxygen transferring substance is strengthened by the fact that, despite its great affinity for oxygen, it remains at a relatively constant level of reduction in the living cell. Willstätter and Mieg,[7] as a result of their work on the oxygen capacity of carotin, arrived at conclusions similar to that of Arnaud. The evidence, however, that carotin may be reversibly oxidized and reduced in cells is as yet very meager. If such evidence can be obtained, the presence of these pigments may be of considerable importance in the oxidative processes in medullated nerves.

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[4] Connor, *J. Biol. Chem.*, 1928, **77**, 619.

[5] Willstätter and Escher, *Z. Physiol. Chem.*, 1910, **64**, 47.

[6] Arnaud, *Compt. Rend. Soc. Biol.*, 1889, **109**, 911.

[7] Willstätter and Mieg, *Ann.*, 1907, **355**, 1.

That portion of the total absorption in the blue end of the spectrum of frog nerves which is not due to carotinoid pigments is probably to be attributed to the partial oxidation of the unsaturated groups in the phosphatids and related substances of the myelin sheath. Commercial (partially oxidized) lecithin, for example, shows a very similar spectrum. *Pure* linoleic acid shows little absorption in this region. If allowed to stand in air, however, general absorption up to about 520$\mu\mu$ soon appears, resulting from the oxidation, and perhaps polymerization of the unsaturated acid.

5491

Changes in Morphology of Giardia Canis as Affected by Diet.

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The study on the *Giardia canis* (Hegner, 1922) of dog revealed the fact that there were at least 2 diverse strains, as has been found with *Giardia* in man.[1] These strains differed one from another with respect to the dimensions and shapes of their cysts and trophozoites. Differences were also noted in the ease of obtaining trophozoites and of producing excystation *in vitro*. Differences in size and shape of cysts remained constant as long as the diets were the same.

When infected animals (young puppies) were kept on a diet rich in carbohydrates, the mean dimensions of cysts were 11.47$\mu \pm .03\mu$ in one and 10.37$\mu \pm .03\mu$ in the other. However, when a carbohydrate diet was replaced by a high protein diet, the respective mean dimensions were found to be 12.05$\mu \pm .03\mu$ and 11.38$\mu \pm .03\mu$. These changes were transitory in nature and were easily reversed by corresponding changes in the diet. In view of the fact that diets affected at the same time change in the bacterial flora from an aciduric to a putrefactive type and vice versa, the question arises as to whether the variations in *Giardia* were due to the changes in diet directly or indirectly as result of changes in the bacterial flora.

With respect to the power to invade the tissues, the strains exhibited no differences—in no instance were there any trophozoites present within the tissues.

Incidentally, in the materials examined during the course of the experiments, there was observed a positive correlation between the lengths and breadths of cysts of the 2 strains, as has been found in the cysts of *Giardia lamblia*,[2] and paralleled the findings of Hegner in regard to *Giardia agilis*[3] and *Trypanosoma diemyctyli*.[4]

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[1] Tsuchiya, H., *Am. J. Hyg.*, 1930, **12**, 467.

5492

Incidence of Protozoan Infections of Intestinal Tract Among Children in Saint Louis.

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This survey was undertaken to determine the relative incidence of the protozoan infections among white children under varying states of health and environment. The subjects were, therefore, taken from 3 distinct groups; the first consisted of 164 inmates of 2 orphanages, the second of 156 patients of 2 hospitals for convalescents and the third of 42 children suffering from variety of acute illnesses. The total was 362 children.

A comparative study of the incidence of protozoan infection as affected by the general state of health showed no significant differences among these groups. In general, the incidence of infection was found to be lowest in the third group (sick children) and the highest among the healthy children in one of the orphanages. This result seems to indicate that environmental conditions play important rôle in the transmission of protozoan infections.

Of the 362 children examined, in 120 cases (33.09%) the infection was limited to one protozoan species; in 37 cases (10.22%) a double infection was found; and in 11 cases (3.02%) there was a triple infection. The incidence of *Giardia lamblia* was the highest, other protozoa following in order of frequency *Endamoeba coli, Chilomastix mesnili, Endolimax nana, Endamoeba histolytica, Iodamoeba williamsi* and *Trichomonas hominis*. In the majority of mixed infections, *Giardia lamblia* was associated with either one or 2 other protozoa. As compared with the surveys conducted elsewhere, the present study revealed one of the highest incidences reported.

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[2] Tsuchiya, H., *Am. J. Hyg.*, 1931, **13**, 544.

[3] Hegner, R. W., *Am. J. Hyg.*, 1922, **2**, 435.

[4] Hegner, R. W., *J. Parasitol.*, 1921, **3**, 105.

The age of children ranged from 1 to 16 years. The highest incidence was found among children between 7 to 9 years old, and the lowest between 1 and 3. The incidence of the infections was much higher among boys than among girls.

Judging from this study, we are inclined to believe that, irrespective of the general state of health of individuals, they may become carriers, if sufficiently exposed to an infection by ingestion of cysts or trophozoites with food or drink. As a matter of fact, among the healthy children in one of the orphanages where the personal hygiene was not sufficiently stressed, the incidence was higher than among sick children temporarily confined in the hospital. On the other hand, in the other orphanage where a rigid hygiene was enforced upon children, there was a very low incidence.

5493

Variations in Optimum pH for Oxidation by Yeast of Substrates, Glucose, Pyruvic Acid and Lactic Acid.

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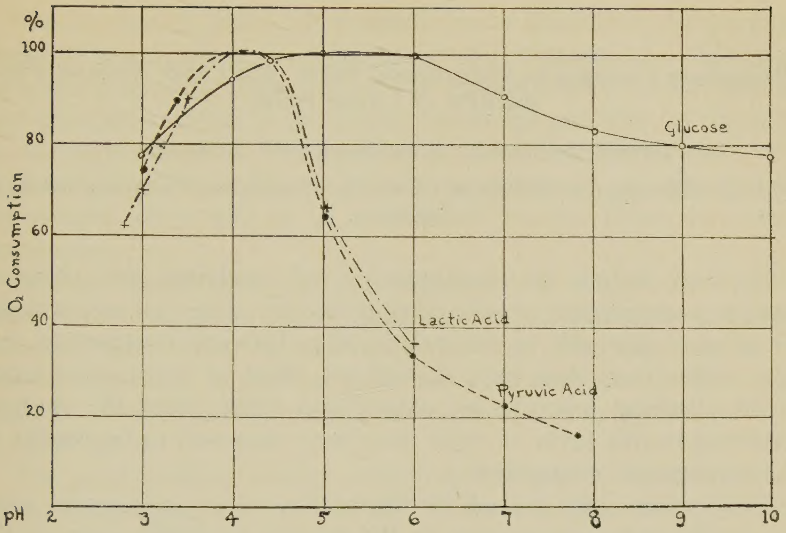
From the Laboratory of Biological Chemistry, Washington University School of Medicine.

The rate of oxygen uptake by yeast in buffer solutions containing the metabolites, glucose, lactate (r.) or pyruvate, is markedly influenced by the buffer pH. The curves of the accompanying figure show average results for 120 minute periods with the 3 substrates at different pH levels. Washed Fleischman yeast, in phosphate, phthalate or citrate buffers of 0.15 M was used with the Warburg manometric apparatus (Temp. 37.5°), the CO_2 being absorbed by NaOH. The pH of the buffer solutions was determined by the quinhydrone electrode, and that of yeast mixtures, colorimetrically.

With glucose as a substrate the maximum of oxygen uptake occurs in buffers having initial pH values from 4.5 to 6.0. With lactate and pyruvate the maximum lies at a pH between 4.0 and 4.5. On the acid side of the optimum, since the oxidation of all 3 substrates is depressed to a similar degree, the glucose curve may be taken as representing the effect of pH on the ability of yeast to oxidize these substances. On the alkaline side of the optimum, the curves of lactic and pyruvic acid fall off more rapidly than that of glucose. The maximum rate of glucose oxidation extends over a



wide range, as was observed by Rona and Grassheim[1] using yeast without added substrate. This difference in the depression of oxygen consumption with different substrates may be related to the fact that beyond pH 4.5, a large part of the pyruvate and lactate are in the form of salts and dissociated as ions, while glucose is in a non-ionized state. The percentage of undissociated acid and salt molecules is approximately proportional to the oxygen consumption at different pH values on the alkaline side of 4.5. These observations might be explained by the inability of ions to penetrate yeast cells.

Changes in pH are accompanied by variation in the completeness of pyruvic and lactic oxidation. From ratios of moles of acid disappearing to moles of oxygen consumed, it can be shown that pyruvic is completely oxidized at its optimum pH, but at a pH of 6 the ratio falls from 2.5 to 1.5 or 1.25. At the optimum of lactic acid oxidation the ratio is 1.5 instead of 3 (showing incomplete oxidation) and falls to 1.25 at a pH of 6, and to 0.734 at a pH of 7.8.

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[1] Rona and Grassheim, *Biochem. Z.*, 1923, **134**, 151.

5494

Phosphate Changes in Chloroform Rigor With and Without Production of Lactic Acid.

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From the Laboratory of Biological Chemistry, Washington University School of Medicine.

The new technic of Lundsgaard[1] for studying the chemical changes occurring in muscle during activity gives an opportunity for a new approach to the relationship between contraction and rigor. We have, therefore, studied the effect of mono-iodoacetate on the chemical processes of chloroform rigor, since the changes occurring in this form of rigor have been supposed to be similar to those of muscle contraction.

The muscles were soaked for 40 minutes in an oxygenated solution of Na mono-iodoacetate in Ringers (1 to 5000); one of the pair was treated with chloroform and the other analyzed for control. The control and experimental muscles were frozen at the same time, thinly sliced and extracted with trichloracetic. On this extract the various phosphate fractions and lactic acid were determined. The changes in the phosphate fractions and in lactic are in the table.

TABLE I.

Treated Muscles.

Values represent changes due to chloroform expressed in mg. per 100 gm.

Muscle used	Rectus	Rectus	Sartorius	Sartorius
Time of Chl.	30	35	60	90
Lactic	+21.0	+30.2	± 0	+58.0
Free P.	+47.0	+41.8	+30.4	+32.9
Creatine P.	—33.9	—29.5	—56.0	— 8.6
Pyro P.	—14.9	—16.3	—10.3	— 8.3
Other Esters	— 6.0	+ 3.9	+25.9	+16.0

Untreated Muscles.

Muscle used	Rectus	Rectus	Gastroc	Gastroc
Time of Chl.	30	40	30	30
Lactic	240.0	416.0	420.0	181.0
Free P.	+20.0	+26.1	+44.8	+20.3
Creatine P.	—32.3	—20.2	—10.7	—32.3
Pyro P.	—10.5	—13.9	—16.3	—11.5
Other Esters	+21.5	+ 8.2	—15.0	+ 9.5

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[1] Lundsgaard, E., *Biochem. Z.*, 1930, **227**, 57.

The decrease in phospho-creatine is more marked in the poisoned muscles than in those in which lactic acid is produced. Admitting the same interpretation as for muscle contraction one must conclude that phospho-creatine is the source of energy and that its resynthesis through the lactic acid mechanism is not interfered with. The high lactic acid values in relation to the extent of phospho-creatine resynthesis show this to be a wasteful process from the energy standpoint.

Analysis made when the muscles have just reached their maximum shortening show little change in the remaining bound phosphorus (other esters in the table) but with time this fraction increases especially in muscles poisoned with mono-iodoacetate. No analysis of this fraction has been made but it is probably carbohydrate ester.

The most striking change occurring in rigor is uninfluenced by mono-iodoacetate, the breakdown of pyrophosphate-adenylic acid. The degree of correspondence between the extent of breakdown of pyrophosphate and the tension of rigor is not adequate to show a quantitative relationship. When rigor is complete 50 to 60% of the pyrophosphate has disappeared. The remainder breaks down within an hour. In mono-iodoacetate rigor, Parnas[2] and Lundsgaard[1] have shown a production of NH_3 accompanying the hydrolysis of pyrophosphate-adenylic acid with the formation of 1 mole of NH_3 to 2 of phosphate. In contraction, although NH_3 is liberated, presumably from pyrophosphate-adenylic acid, either the course of reaction is different or resynthesis occurs immediately, since there is little change in this phosphate fraction, unless stimulation proceeds to extreme fatigue anaerobically. The breakdown of pyrophosphate-adenylic acid appears to be the result of the processes causing rigor rather than the cause of rigor itself, unless the inosinic acid formed is more toxic than NH_3 or phosphate.

Iodo-acetic acid rigor occurs in muscles, after stimulation, when phospho-creatine has been completely broken down and when no lactic acid is being formed. It is therefore different from chloroform rigor, or the hydrolysis of phospho-creatine is incidental to the mechanism as is lactic acid.

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[2] Parnas, J. K., *Naturwissen,* 1930, **44**, 916.

5495

Effects of Crystalline Theelol and Theelin and Extracts of Liquor Folliculi on Male Rats.

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Gould and Doisy[1] found that the subcutaneous administration of extracts of hog liquor folliculi retarded the development of the testes of young rabbits. Laqueur[2] has obtained results of a similar nature by the injection of young rats with Menformon, and Moore and Price[3] have found that oestrin produces testicular damage. Spencer, Gustavson and D'Amour[4] report that the administration of oestrin obtained from pregnancy urine retards the rate of growth and testes development of young male rats.

The isolation of theelol[5, 6, 7] led us to compare the effects of daily injections of crystalline theelin and theelol and extracts of hog liquor folliculi upon the gain in body weight and the development of the genitalia of young male rats. This report is concerned with changes of body and testes weight; a further study including microscopic findings will be presented in a later paper.

Subcutaneous injections of from 1.25 to 20 rat units (Kahnt and Doisy[8]) were made daily for 19 days in normal male rats 21 to 30 days of age. The preparations were aqueous solutions containing from 1 to 5% ethyl alcohol. Only litter mate controls were used but in a few cases one control served for 2 experimental animals. The controls were injected with a similar volume of alcohol of the same concentration. All animals were kept under the same conditions and were fed *ad libitum* upon the same adequate diet.

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[1] Gould, V. L., and Doisy, E. A., Observations (1924) not published.

[2] Borchardt, E., Dingemanse, E., deJongh, S. E., and Laqueur, E., *Zeit. f. Ges. Exp. Med.*, 1929, **68**, 86.

[3] Moore, C. R., and Price, Dorothy, PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 38.

[4] Spencer, J., Gustavson, R. G., and D'Amour, F. E., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 500.

[5] Doisy, E. A., Thayer, S. A., Levin, L., and Curtis, J. M., PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 88.

[6] Doisy, E. A., Curtis, J. M., Levin, L., Katzman, P. A., and Thayer, S. A., PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 216.

[7] Doisy, E. A., and Thayer, S. A., *J. Biol. Chem.*, in press.

[8] Kahnt, Lydia C., and Doisy, Edward A., *Endocrinology*, 1928, **12**, 760.

Twenty-seven of the 36 experimental animals gained less in body weight than their controls. The average values are summarized in Table I.

TABLE I.

	Theelol					
	Rat Units Daily, 1.25			5.00		
	No. rats used	Av. gain body wt.	Relative % testes wt.	No. rats used	Av. gain body wt.	Relative % testes wt.
Controls	6	75	1.00	6	77	1.00
Experimentals	7	69	.80*	6	64	.88
	Theelin					
	Rat Units Daily, 5			20.00		
Controls	5	77	1.00	5	84	1.00
Experimentals	8	69	.87	7	72	.53
	Liquor Folliculi					
	Rat Units Daily, 5					
Controls	7	70	1.00			
Experimentals	8	62	.63			

* Six rats used here.

Since the testes weight is dependent in part on the body weight, the ratio of testes weight to body weight was taken and from the values of the injected and control animals the relative percentage weight was calculated, taking the control as unity. Of a total of 35 experimental animals 32 were below their controls in testes development. The average values appear in the table.

Minnesota Section.

University of Minnesota, March 18, 1931.

5496

Proportions of the Various Constituents of the Normal Adult Human Female Hypophysis.*

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This note on the weight of the normal adult human female hypophysis is based on serial sections of 43 non-pregnant women, 18 to 40 years old; 20 pregnant individuals, 15 to 40 years of age, and 27 older women, 50 to 81 years. The data were obtained from the absolute weight of the whole organ (after removal of the stalk close to the main body of the gland and therefore not including the stalk and *pars tuberalis*), and the relative weight of its parts obtained by projecting every 20th section on paper and then cutting out and weighing the areas.

The mean weight of the whole gland (less capsule, stalk and *pars tuberalis*) of young adult non-pregnant women is 620 mgm., with a minimum weight of 483 mgm. and a maximum weight of 971 mgm. This is 13% higher than the weight of the male hypophysis of similar age. Undoubtedly this is due to some of the females having been pregnant one or more times, since the literature indicates that the hypophysis of nullipara is essentially the same in weight as that of the male. Our figures are not directly comparable to those in the literature on account of having eliminated from our specimens all the surrounding connective tissue, which is an extremely variable quantity, having nothing to do with the essential constituents of the organ.

The hypophysis of pregnant individuals varies from 528 mgm. to 1111 mgm., the mean being 731 mgm., which is 18% greater than that of the non-pregnant group of the same age, and 17%

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* Aided by a grant from the Research Fund of the Graduate School, University of Minnesota, and from the Grant-in-Aid Fund of the National Research Council.

greater than the hypophysis of old women. This enlargement is distinctly less than one would expect from the literature, probably due to a preponderance of early stages of pregnancy in our material. Although all stages of pregnancy are represented, we do not have enough data to determine the correlation of the weight of the hypophysis with the duration of pregnancy; but judging from less accurate weights on record, there is a progressive increase towards the time of parturition.

The hypophysis of females from 50 to 81 years of age varies from 478 mgm. to 803 mgm., the mean being 625 mgm., which is 15% less than in the pregnant group, and 25% more than in old men. Evidently the hypertrophy of pregnancy tends to persist into old age.

The larger size of the female hypophysis as compared with the male, and of the pregnant as compared with the non-pregnant female, is due to a larger anterior lobe. The posterior lobe is slightly smaller in females than in males of the same age and especially so in the case of pregnant females. This results in a distinctly higher relative weight of the anterior lobe in the female hypophysis. This is most marked in the pregnant female where the anterior lobe averages 85% (as compared with about 75% in the male) of the entire gland, leaving 14% for the posterior lobe, and less than 1% for *pars intermedia* (including the colloid in the residual lumen).

The posterior lobe of the female shows a slight absolute increase in old age just as occurs in males.

The mean value of *pars intermedia,* which in the male is in the neighborhood of 2% of the entire gland, is distinctly smaller in pregnant and old women than in the male and young adult non-pregnant females. This is particularly true of the amount of colloid. The parenchyma of *pars intermedia* increases distinctly in old women, but not to the extent that occurs in old men. There is no ponderal or obvious histological evidence in support of the supposed increased activity on the part of *pars intermedia* during pregnancy.

5497

Normal Osmotic Pressure of the Plasma Proteins of Man.*

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From the Department of Medicine, University of Minnesota, and the Minneapolis General Hospital.

There have been many determinations of the "normal" osmotic pressure of the plasma proteins of human blood, namely, Starling,[1] Govaerts,[2] Krogh,[3] Schade,[4] Verney,[5] and Landis.[6]

It is our opinion that, with the exception of Schade's determinations, there is some possibility of considerable error in all of these determinations because the determinations were made from 12 to 48 hours after taking the blood from the veins, during which time an experiment carried out by us shows that peptization of the proteins can take place, thus increasing the number of colloid particles and the colloid osmotic pressure. In some of these measurements no attempt was made to keep the temperature constant during the period of measurement and equilibration. Moreover, fluids were used on the outside of the osmometer membranes which were not exactly those of the dispersion medium. Schade[7] has devised a method which we believe is accurate to 5%. It is based upon a method devised by Sorenson.[8] Determinations can be made by Schade's method in 15 minutes after temperature equilibrium has been reached, thus largely avoiding the danger of peptization. Moreover, temperature is kept constant to within 0.02-0.03° in a properly constructed bath during the period of measurement. In this method no solution of salts is placed upon the outer side of the semipermeable membrane (collodion). The plasma is put under a measured pressure and the rate of ultrafiltration is determined, then the pressure is changed and the rate of ultrafiltration is again determined. A curve of rate of filtration as ordinates and pressures as abscissae is constructed from these values. This is always a straight

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*Carried out with the aid of a grant from the Ella Sachs Plotz Foundation.

[1] Starling, E. H., *J. Physiol*, 1899, **24**, 317.

[2] Govaerts, P., *Bull. d. l'Acad. Roy. d. Med. d. Beligigne.*, 1924, **4**, 161.

[3] Krogh, A., *Anat. and Physiol. of the Capillaries*, Yale Univ. Press, New Haven, 1922.

[4] Schade, H., *Ergeb. d. Inn. Med. Kinderh.*, 1927, **32**, 425.

[5] Verney, E. B., *J. Physiol.*, 1926, **61**, 319.

[6] Landis, E. M., *Am. J. Physiol.*, 1930, **93**, 353.

[7] Schade, H., *Z. f. Klin. Med.*, 1924, **100**, 370.

[8] Sorenson, *Compt. Rend. d. Trav. du Laboratoire de Carlsberg*, 1917, **12**, 262.

line. The pressure corresponding to the point where this curve crosses the line of zero filtration rate is the osmotic pressure of the colloids which can not pass the collodion membrane. It must be remembered that the electric charge on the membrane has an influence on osmotic pressure and filtration rate. The values determined by this method, therefore, relate to a collodion membrane only.

Using Schade's method we have determined the osmotic pressure of the plasma proteins† for 6 normal persons.

TABLE I.

Osmotic Pressure of Plasma Proteins of Normal Human Blood at 37°C. (against collodion membrane).

Name	Colloid Osmotic Pressure in mm. Hg.	Plasma Proteins
		%
W. S.	20	
W. C.	20	9
E. G.	21	8.2
R. H.	25	6.6
E. G.	20	8
V. A.	20	
P.	23	6.4
Mean	21	

Landis[9] has shown that the pressure at the beginning of the human skin capillaries is about 32 mm. Hg. At the middle it is about 20 mm. Hg. and at the venous end it is about 14 mm. Hg. Our figures agree well with his "average" value for the middle of the skin capillaries. Values of osmotic pressure of plasma proteins above 30 mm. Hg. would seem too high for they would not allow of filtration of water out of capillaries into the tissue excepting in the glomerulus of the kidney.

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† 0.4% sodium citrate was added to the plasma to prevent coagulation.

[9] Landis, E. M., *Heart*, 1930, **15**, 209.

5498

Osmotic Pressure of Plasma Proteins in Nephritis.*

GEORGE FAHR, ARTHUR KERKHOF AND CLAIRE CONKLIN.

From the Department of Medicine, University of Minnesota, and the Minneapolis General Hospital.

We have determined the osmotic pressure of the plasma colloids in normal persons and in cases of glomerulonephritis with and without edema, using the method of Schade[1] for the determination of the osmotic pressure of the plasma proteins at 37°C. against a collodion membrane. The normal values for the osmotic pressure of the plasma proteins were from 20 to 23 mm. Hg. The osmotic pressure of the plasma proteins was 11 mm. Hg. or lower in all our cases of nephropathic edema investigated up to the present time. Edema was not observed in cases of nephritis where the osmotic pressure of the colloids of the plasma was 17 mm. Hg. or above. Undoubtedly lowering of the osmotic pressure of the plasma proteins is a factor in the formation of edema in nephritis and nephrosis. It is best thought of as determining a "tendency to edema formation." Other factors as ability of the kidneys to excrete water, salt retention,

TABLE I.
Osmotic Pressure of Plasma Proteins in mm. Hg. at 37°C. in Nephritis.

Name	Diagnosis	Osmotic Pressure of Plasma Protein in mm. Hg. at 37°C.	Serum Albumin %	Plasma Proteins %	Edema
S. M.	Sub-chronic Glomerulo-nephritis	6		3.7	Present in large amount
S. M.	,,	9			Present
S. M.	,,	8		3.4	,,
S. M.	,,	10		4.6	,,
S. M.	,,	9	2	4.4	,,
S. M.	,,	17	3.1	4.8	None
D. G.	,,	9	2.3	4.1	Present
E. W.	Nephrosis	10			,,
D. H.	Chronic Glomerulonephritis	9		3.9	,,
D. H.	,,	10	1.8	3.8	,,
D. C.	Amyloid kidney	11	1.3	4.7	,,
O. M.	Sub-chronic Glomerulo-nephritis	10	2	4.1	,,
L. J.	Chronic Glomerulonephritis in uremia	27	4.4	6.4	None

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* Carried out with the aid of a grant from Ella Sachs Plotz Foundation.

[1] Schade, H., *Z. f. Klin. Med.*, 1924, **100**, 370.

anoxemia and toxins altering capillary permeability, etc., contribute to the formation of edema. By reducing water intake it was possible to reduce the extent of the edema in some cases with low osmotic pressure of the plasma proteins. We are now attempting to determine experimentally the level of osmotic pressure of the plasma proteins below which edema must form even with good renal function and no salt retention. When the osmotic pressure of the plasma proteins is below the hydrostatic pressure at the venous end of the capillary system, undoubtedly edema must form because water can not stay in the capillaries.

Our figures are in essential agreement with those obtained by Schade,[2] Govaerts,[3] and Krogh[4] on similar cases.

5499

A Method for Preparing an Active Adrenal Cortex Extract.

R. L. ZWEMER, F. J. AGATE, JR., and H. A. SCHROEDER.
(Introduced by P. E. Smith.)

*From the Department of Anatomy, College of Physicians and Surgeons, Columbia University.**

A number of investigators have recently[1, 2] reported active extracts of the adrenal cortex, but at the time we were ready to use cortical extracts on test animals we were unable to find detailed directions for their preparation. As a result we have developed the following method.

Beef adrenals obtained from the slaughter house immediately after the kill are transported to the laboratory, packed in ice. The connective tissue and fat are dissected off, and the glands ground in a meat chopper equipped with a fine cutting disc. The minced glands are weighed and placed in 95% ethyl alcohol, one liter of alcohol per kilogram of gland, and allowed to stand in the cold over night. The next morning the alcohol is poured off, employing a coarse cheese cloth as a strainer. The alcohol is then filtered through a coarse filter paper and the filter washed with fresh alcohol. This alcohol, together with the washings, is saved.

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[2] Schade, H., *Ergeb. d. Inn. Med. Kinderh.*, 1927, **32**, 425.

[3] Govaerts, P., *Bull. d. l'Acad. Roy. d. Med. d. Beligigne.*, 1924, **4**, 161.

[4] Krogh, A., *Anat. and Physiol. of the Capillaries*, Yale Univ. Press, New Haven, 1922.

* We wish to thank the Department of Biochemistry for their generosity in allowing us the use of special equipment and reagents.

[1] Swingle, W. W., and Pfiffner, J. J., *Science*, 1930, **71**, 321.

[2] Hartman, F. A., *Endocrinol.*, 1930, **14**, 229.

The adrenals are placed in a continuous extraction apparatus and extracted with alcohol for 84 hours under reduced pressure, and at a temperature between 35°C. and 40°C. After extraction the alcohol extract is combined with the alcohol in which the glands were dehydrated and placed in the cold for 12 hours, during which period a white precipitate settles out. The extract is then filtered and concentrated to half its volume under reduced pressure and again filtered. The remaining alcohol is removed under reduced pressure and the aqueous residue placed in the cold for 24 hours. This extract is centrifuged in ice at a speed of 2000 R.P.M. for 10 minutes. A light brown, slimy precipitate settles out and a few drops of orange colored oil appear on the surface of the supernatant fluid; the supernatant fluid is withdrawn with a pipette and its equivalent in grams of gland computed. It may be passed through a Seitz filter for purposes of sterilization.

Further purification is obtained by shaking the supernatant fluid with an equal quantity of benzene in a separatory-funnel. The benzene fraction is freed from the benzene by evaporation under reduced pressure over a small quantity of water. The aqueous residue is extracted with ether. The ether fraction is shaken up with distilled water and the ether then removed by reduced pressure. The ether-free aqueous solution is the potent extract and is diluted with water when needed for injection. It is adrenin-free by the Folin-Denis phenol reagent.

The activities of the various fractions obtained during this procedure were tested by their effect on blood constituents of normal and adrenalectomized cats, as well as their effect on the general health, weight and appetite of the animals. The results are described in a following paper by Zwemer and Sullivan.[3]

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[3] Zwemer, R. L., and Sullivan, R. C., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 723.

5500

A Blood-Chemical Test for Adrenal Cortex Extracts.

R. L. ZWEMER AND R. C. SULLIVAN. (Introduced by P. E. Smith.)

From the Departments of Anatomy and of Diseases of Children, College of Physicians and Surgeons and the Babies' Hospital, Columbia-Presbyterian Medical Center.

The clinical picture of adrenal insufficiency in animals has been described by many authors. Certain of the blood changes have also been described, but for the most part these have been isolated or terminal findings. Our experiments have extended the chemical studies to day by day observations. We find that a fall in CO_2 capacity, a non-protein nitrogen rise, a chloride fall and a fall in blood sugar occur in experimental adrenal insufficiency. The blood-chemical picture has been correlated with observed symptoms of loss of appetite, asthenia, dehydration, and decreased peripheral circulation.

Injection of our adrenin-free extract of the adrenal cortex,[1] brings about a reversal of the shift in adrenal insufficiency. The CO_2 capacity and blood chlorides rise to normal, the non-protein nitrogen fails to rise, and the blood sugar increases. There is in adrenalectomized cats an immediate clinical improvement and their appetite for solid food returns. A few animals injected while in a comatose state, have soon after gotten up and walked around.

Even normal animals react to doses of this cortex extract and we use them to test its potency. The injection of 0.5 cc./kg. body weight of an extract in which 1 cc.=0.5 gm. of fresh whole beef adrenal, will produce a rise in carbon-dioxide capacity and in blood sugar. A fall occurs in the non-protein nitrogen, if it is high, and a rise, if it is normal. The same shift is found in the blood chlorides. Similar results have been obtained using cats, dogs and rabbits.

The maximal chemical changes are not immediate. The blood sugar reaches its high point in 1 to 5 hours; the chlorides are changed within an hour or so; the rise in carbon-dioxide capacity, which is the most significant change, occurs in about 5 hours, but takes 24 to 48 hours to reach its peak. Injections of preparations not adrenin-free produce an initial drop in the carbon-dioxide combining capacity.

The use of normal animals for tests has obviated the necessity for many adrenalectomies and also conserves material, since we and others[2, 3] have found that it requires huge quantities of extract to keep adrenalectomized cats alive.

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[1] Zwemer, R. L., Agate, F. J., and Schroeder, H. A., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 721.

As yet a lack of uniformity in test animals must be encountered. An animal with an initial low carbon-dioxide capacity will show a greater reaction to the extracts than one injected when the carbon-dioxide capacity is high.

Forty-two adrenalectomized and 51 normal cats form the basis for this paper. Tests were repeatedly made on certain of these animals. Over 400 blood chemical determinations were made on the above animals. In studying the clinical symptoms of adrenal insufficiency over 200 animals were used.

5501

Continuous Pancreatic Secretion.

B. N. BERG AND T. F. ZUCKER.

From the Department of Pathology, College of Physicians and Surgeons.

The following study was undertaken in order to determine the characteristics of the flow of pancreatic juice in the fasting dog and the influence of certain stimuli upon the rate of pancreatic secretion. Cl. Bernard[1] and Pavlov[2] believed that pancreatic juice was secreted intermittently in response to specific stimuli. Continuous secretion was regarded by them as an abnormal response of the pancreas to irritation and inflammation. Farrell and Ivy[3] found that pancreatic transplants secreted continuously in dogs. Recently Baxter[4] reported that spontaneous pancreatic secretion occurred in rabbits under prolonged urethane anesthesia, after decerebration or decapitation, and after extirpation of the entire gastro-intestinal tract.

Permanent pancreatic fistulas were established in 4 dogs under ether anesthesia and with strict asepsis according to the technic described by Elman and McCaughan.[5] This method permitted a study of pancreatic secretion under relatively normal conditions. The pancreatic juice remained uncontaminated, the total output being collected and measured at 24 hour intervals. The animals were allowed to recover completely from the effects of the operation before observations were made. For the first 2 days after the establishment of the fistula, secretion was either absent or markedly diminished. The experiments were conducted 4 to 8 days after the onset of secretion. Autopsy examinations were negative. The pancreas showed no gross or microscopic evidences of inflammation or obstruction. The gastro-intestinal tract was found to be empty at the conclusion of the fasting experiments.

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[2] Hartman, F. A., *Endocrinol.*, 1930, **14**, 229.

[3] Swingle, W. W., and Pfiffner, J. J., *Science*, 1930, **71**, 321.

[1] Bernard, C., *Mémoire sur le pancréas.* 1856.

[2] Pavlov, I. P., The work of the digestive glands. London, C. Griffin and Co., 1910.

[3] Farrell, J. I., and Ivy, A. C., *Am. J. Physiol.*, 1926, **78**, 325.

[4] Baxter, S. G., *Am. J. Physiol.*, 1931, **96**, 343.

The rate of secretion was studied by the "drop method," 14 drops of pancreatic juice being equal to 1 cc. Observations were made at 1 minute intervals for 60 minute periods. The secretory pressure was determined by measuring the maximum height attained by the column of juice when the duct system was connected with a manometer 2 mm. in diameter. A column 100 mm. in height was equivalent to 0.7 cc. of pancreatic fluid. For practical purposes the manometric readings were interpreted as indicators of secretory pressure although it was recognized that there were factors in the secretory system, viz., elasticity of the duct walls, changes in vascularity of the pancreas, etc., which were probable sources of error. Since the conditions under which the experiments were performed remained relatively constant and since the difference between the pressure during fasting and during digestion was beyond normal variations, the determinations were considered significant.

Preliminary to the study of pancreatic secretion during fasting, the dogs were deprived of food and water for a minimal period of 24 hours. In order to avoid the possible influence of conditioned reflexes upon secretion, the animals were observed in a room where they were not accustomed to receive food. They were trained to lie quietly and often slept while the experiments were in progress.

The average rate of pancreatic secretion determined by the "drop method" in 2 fasting dogs was 2.8 drops per minute or approximately 12 cc. per hour. These figures coincided approximately with the hourly output of juice calculated upon a 24 hour basis, the total volume secreted per day varying between 250 cc. and 350 cc. The maximal secretory pressure in 2 fasting animals varied between 300 mm. and 340 mm. These levels were attained within periods of 12 minutes to 15 minutes.

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[5] Elman, R., and McCaughan, J. M., *J. Exp. Med.*, 1927, **45**, 561.

When 10 cc. of a solution of secretin[6] was injected intravenously into a fasting dog, the flow of pancreatic juice was increased temporarily from an hourly rate of 2.8 drops per minute to 4 drops per minute. This acceleratory effect lasted 10 minutes[7] and then the regular fasting rate of secretion was resumed. During digestion a prolonged acceleration occurred. Four hours after a meal consisting of meat the secretory pressure was 380 mm. in contrast to a previously fasting level of 300 mm.

Summary. Pancreatic secretion is continuous in fasting dogs. The rate of secretion is accelerated after the injection of secretin and during digestion. When the effects of these stimuli subside the flow of pancreatic juice continues.

5502

Action of Thyroxin on Tissue Metabolism.

ALEITA HOPPING. (Introduced by E. L. Scott.)

From the College of Physicians and Surgeons, Columbia University.

Magnus-Levy[1] showed that the thyroid gland increased metabolism. The manner in which this effect is brought about is still a matter for discussion.[2, 3] Adler[4] stated that thyroid action persisted after ergotamine and concluded a peripheral action. However, Abderhalden and Wertheimer[5] found that ergotamine inhibited the effect of a previous thyroid injection. The great amount of work done on the direct effect of thyroxin on tissue metabolism has served to complicate rather than to clarify matters. Fleischmann,[6] Rothschild,[7] Simon,[8] Paasch and Reinwein,[9] Anselmino,[10]

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[6] Weaver, M. M., Luckhardt, A. B., and Koch, F. C., *J. Am. Med. Assn.*, 1926, **87**, 640.

[7] Bayliss, W. M., and Starling, E. H., *J. Physiol.*, 1902, **28**, 325.

[1] Magnus-Levy, *Berl. klin. Wschr.*, 1895, **32**, 650.

[2] Plummer, *J. Am. Med. Assn.*, 1921, **77**, 243.

[3] Kendall, *Tr. Ass. Am. Phys.*, 1918, 324; Kendall and Simonson, *J. Biol. Chem.*, 1928, **80**, 357.

[4] Adler, cited by Reinwein, Helmuth and Singer, *Biochem. Z.*, 1928, **197**, 152.

[5] Abderhalden and Wertheimer, *Pflüger's Arch.*, 1927, **216**, 697.

[6] Fleischmann, *Biochem. Z.*, 1930, **187**, 324.

[7] Rothschild, *Biochem. Z.*, 1930, **217**, 365.

[8] Simon and Wiener, *Biochem. Z.*, 1929, **215**, 102.

[9] Paasch and Reinwein, *Biochem. Z.*, 1929, **211**, 468.

[10] Anselmino, Eichler and Schlossman, *Biochem. Z.*, 1929, **205**, 481.

Ellinger,[11] obtain negative results with the thyroid principle, while Wohlgemuth and Klopstock,[12] Weil,[13] and Ahlgren[14] find an increase in metabolism.

The method used in this work consists in placing (alligator) blood (6 to 10 cc.) in duplicate tonometers containing air under alveolar tensions (CO_2 3%, O_2 17%, in the alligator) and measuring by direct analysis of the pair, the O_2 and CO_2 contents at the beginning and end of a test period, maintaining the tonometers in a constant temperature bath. The gas phase was analyzed by the Haldane apparatus, the liquid phase by the Van Slyke manometric method of blood gas analysis. Calculating the volumes of oxygen consumed and carbon dioxide liberated, under standard conditions of temperature and pressure, allows the expression of the metabolism of the blood in terms of small calories* per hour per 1000 cc. of blood (with 500,000 red cells per cu. mm.). The work was done with bacteriological precautions, cultures of the blood being

TABLE I.

No. of Exp.	Temp. °C.	R. Q.	Small Cal.*	Character of Experiment	% change
21	25	1.30	10.9	Control	
22	25	0.72	11.6	Addition of thyroxin (3×10^{-6} g. per 1 cc. of blood)	
24	25	0.68	15.1	Control	
31	30	1.08	22.0	”	
32	30	0.99	21.8	Addition of thyroxin (7×10^{-6} g. per 1 cc. of blood)	—0.1
33	30	0.53	32.1	Injection of thyroxin 7×10^{-4} g.	+46
35	30	1.80	14.2	Incubation (7×10^{-6} g. in 1 cc.)	No change
36	30	0.90	14.4	Control	
37	30	0.70	13.9	”	
38a	30	1.16	18.6	Addition (6×10^{-6} g. in 1 cc.)	+17
39	30	0.99	15.7	Incubation (3×10^{-6} g. in 1 cc.)	+10
40‡	30	0.81	24.0	Control	
41†	30	0.70	23.6	Injection (3×10^{-3} g.)	No change
43	30	0.86	68.8	” (4.5×10^{-3} g.)	+183
44	30	0.83	60.8	” ”	+153
46	30	0.33	25.1	Addition of thyroxin (4×10^{-5} g. per cc. of blood)	+ 5
47	30	0.70	21.2	Addition of thyroxin (1×10^{-3} g. per cc. of blood)	— 10

*Small calory, the amount of heat necessary to raise the temperature of 1 gm. of water 1°C.

†Experiments No. 41 and 43 were run on the same animal.

‡Oxalate in isotonic concentration was used as an anticoagulant in previous experiments, heparin in this and later ones.

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[11] Ellinger, *Z. f. phys. Chem.*, 1922, **119**, 11.

[12] Wohlgemuth and Klopstock, *Biochem. Z.*, 1926, **175**, 202.

[13] Weil and Landsberg, *Biochem. Z.*, 1924, **207**, 186.

[14] Ahlgren, *Skan. arch. f. Phys.*, 1925, **47**, supp. 225.

plated out at the end of the experiment, as a routine procedure, retaining only those showing negative results.

We find by this method of measuring tissue metabolism that the blood from an alligator injected with thyroxin (Squibbs) 3 days or so previously to the test may show an increase of from 150 to 190% above the control. Direct addition of thyroxin to the blood, on the other hand, has produced no change which has an interpretable significance.

We have been unable to demonstrate that incubation of thyroxin in the blood *in vitro* has an activating effect.

Further investigation is contemplated to confirm this metabolic increase in the tissues of the injected animal and to determine the mechanism by which it is brought about.

The considerable variations among the controls are apparently due to the different seasons, different laboratory conditions, different animals used, successive use of the same animal.[15]

5503

Effect of Adrenal Cortical Hormone Upon Respiratory Metabolism of Adrenalectomized Cats.*

W. W. SWINGLE, J. J. PFIFFNER AND BRUCE WEBSTER.

From the Department of Medicine, College of Physicians and Surgeons, Columbia University, and Princeton University.

The material here presented represents an attempt to determine the effect of bilateral adrenalectomy on the respiratory exchange of cats, together with the changes in metabolism produced by the administration of the cortical hormone to these adrenalectomized animals. There is, in the literature, some confusion as to the effect of bilateral adrenalectomy on the metabolism of animals. Golyakowski[1] reported an increase in heat production after ligation of the blood supply of the adrenals in dogs. Marine and Baumann[2] reported an increase in metabolism in 53% of their rabbits where the adrenals were removed or injured by freezing. They noted no change in 33% and a fall in 14%. Scott,[3] using cats, obtained a slight increase in some animals and no change in others after subtotal injury of the adrenal glands, and a fall in metabolism following total removal. Aub,[4] also using cats, found a drop of 25% in the metabolism, following total removal of the adrenal glands. He did, however, detect a slight increase immediately following operation. Intravenous injection of the adrenal cortical extracts available at that time (1922) produced no effect on the metabolism of these animals.

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[15] Barron and Harrop, *J. Exp. Med.*, 1928, **48**, 207.

* This investigation has been aided by grants from the Josiah Macy, Jr., Foundation and Mrs. R. E. Breed.

[1] Golyakowski, *Vrach.*, St. Petersburg, 1899, **20**, 1017.

[2] Marine, D., and Baumann, E. J., *Am. J. Physiol.*, 1921, **57**, 135.

Following the introduction of an active extract of the adrenal cortex by Swingle and Pfiffner[5] it was considered advisable to determine its effect on the metabolism of adrenalectomized cats. The operation was performed in 2 stages in the following experiments. Healthy adult animals were used. They were maintained on a constant diet and were fed approximately 20 hours before the beginning of the metabolism determination. The respiratory exchange was measured by Marine's modification of Haldane's open circuit apparatus[6] for two-hour periods. Injections of the cortical hormone were made at least 15 hours prior to the beginning of the determination of metabolism. The method of preparation of the cortical hormone used was that described in detail by Swingle and Pfiffner.[7] The adrenalin content of this extract was between one part in 1,500,000 and one part in 2,000,000. The injections were made subcutaneously.

Previous observers[2] have shown that the removal of one adrenal does not alter the level of metabolism. Following the removal of the second adrenal gland, there was no appreciable change in metabolism for from 4 to 6 days in our series of animals. From the sixth to the tenth day there was a progressive fall until a level approximately 50% below normal was reached when the animal was in a prostrate condition. Following the administration of cortical hormone to these prostrate animals, an increase in metabolism occurred within 24 to 48 hours. In from 48 to 72 hours after the beginning of the administration of the hormone, the metabolism had risen, in each case, to a level varying between 10 and 18% above the normal previously established for that individual animal. At this point the administration of hormone was stopped and there was a fall within 24 to 36 hours to the normal level. This was maintained for a varying time period (several days), during which the animal appeared normal. At the end of that time the metabolism began gradually to decrease, signs of adrenal insufficiency developed, and the cycle was repeated.

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[3] Scott, W. J. M., *J. Exp. Med.*, 1922, **36**, 199.

[4] Aub, J. C., Forman, J., and Bright, E. M., *Am. J. Physiol.*, 1921, **55**, 293.

[5] Swingle, W. W., and Pfiffner, J. J., *Anat. Rec.*, 1929, **44**, 225; *Am. J. Physiol.*, 1931, **96**, 153.

[6] Marine, D., *J. Metabolic Research*, 1922, **2**, 29.

[7] Pfiffner, J. J., and Swingle, W. W., *Am. J. Physiol.*, 1931, **96**, 180.

The changes have been studied in a series of 6 cats. One was observed through 3 cycles, one through 2 and the remainder through one. The metabolic changes were essentially the same in each case.

Further observations are in progress as to the effect of the cortical hormone on normal animals, and an attempt is being made to determine the rôle of the thyroid gland in the experiments described above.

5504

Solubility of Human Gall Stones in Dog Bile.

LOUIS BAUMAN AND GERHARD O. SPANNER.

From the Chemistry Laboratory, Department of Surgery, Columbia University, and Presbyterian Hospital.

That human gall stones dissolve in gall bladder bile of the dog has aroused considerable interest over a period of years. The reason for this phenomenon has never been accurately determined. Recently Walsh and Ivy[1] showed that the unsaponifiable fraction of dog bile which includes cholesterol, is smaller than that of human bile, while the saponifiable matter, which contains bile salts and soaps, is considerably larger. No quantitative estimation of the individual bile constituents was made.

The concentration of cholesterol in gall bladder bile of the human, beef and dog was determined by the gravimetric method of Windaus, *before* and *after* saturation with finely powdered cholesterol. This sterol is easily the most important constituent of human gall stones from the quantitative aspect. The figures in Table I indicate that while dog and ox bile contain little cholesterol they are capable of dissolving considerable quantities of this substance; this, we believe, is the chief cause for the solubility of human gall stones in gall bladder bile of the dog.

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[1] Walsh, E. L., and Ivy, A. C., *Ann. Int. Med.*, 1930, **4**, 134.

TABLE I.
Analytical data of gall bladder bile.

Spec. No.	Solid Matter %	Cholest. mg. %	Cholest. / Solids %	Cholest. after saturation mg. %	Increase in Cholest. %
			Human Bile		
608	4.95	112	2.26	133	18.7
612	15.0	364	2.42	408	9.3
613	18.0	610	3.38	800	31.0
614*	4.75	86	1.85	103	20.0
			Dog Bile		
63	24.79	70	.28	180	157.0
64	25.02	130	.52	320	146.0
56	22.0	8	.03	134	1675.0
57	15.0	8	.05	96	1200.0
58	22.8	2	.01	288	14400.0
59	16.7	2	.01	428	21400.0
72†	21.28	2	.01	415	20750.0
73	18.92	20	.10	70	350.0
75	23.8	82	.34	208	253.0
84	16.47	44	.27	138	313.0
			Ox Bile		
74	10.76	43	.40	124	288.0

* Mixture of 30 autopsy biles diluted with water.
† Mixture of 2 specimens.

5505

Amino Acids as the Only Nitrogen Source During Growth and Differentiation.

O. HOFFMAN and F. GUDERNATSCH. (Introduced by A. J. Goldforb.)

From New York University, Washington Square College.

Experiments are in progress on forms lower than mammals to determine the value of isolated amino acids for protein synthesis and to ascertain which acids if any are essential for, or facilitate the growth of organisms of different phylogenetic levels, and which are of importance in the process of differentiation. An attempt has been made to bring tadpoles of *Rana sylvatica* to metamorphosis on a diet in which the only N available was the N of isolated amino acids. Carbohydrates, fats and vitamins were supplied in the form of a "Basal Diet". The following amino acids were used in concentrations of 1% and 5%, added to the water: glycine, alanine, leucine, aspartic, glutamic, arginine, lysine, cystine, phenyl-alanine, tyrosine, tryptophane, proline, diiodo-tyrosine. Single acids and bi-acid mixtures were used, each acid being combined with every other. Furthermore, a progressive series was arranged, one acid at a time being added to the previous mixture, until all 14 had been introduced.

The Growth Factor. Arginine was a good acid for growth, while signs of differentiation were very meagre in the arginine groups. Cystine proved to be a very good growth factor, closely following arginine. Phenyl-arginine acted very favorably on growth, especially when combined with another acid suitable for maintenance or growth. In combination with arginine or cystine, phenyl-alanine acted particularly well. Lysine also favored growth, histidine hardly so.

The Differentiation Factor. Phenyl-alanine and 2 other acids containing aromatic rings, tyrosine and tryptophane, belong in this group. A weak differentiation factor is present, *e. g.,* in phenyl alanine; the number of animals showing significant differentiation was high (50%); in tyrosine, 9 out of 14 cultures showed signs of differentiation; in tryptophane, a few animals showed advanced differentiation.

The Maintenance Factor. The maintenance and growth factors do not coincide in every case. Leucine is a very good acid for maintenance, not for growth. Alanine is fairly good for maintenance only. Arginine causes growth, maintaining the animals fairly well. Cystine is good in both respects. Histidine is extremely poor for maintenance, aspartic still more so. The maintenance factor is expressed plainly in the mortality rates. In tryptophane (differentiation acid), the first death and the majority of deaths occur early and close together; in arginine (growth acid), they occur later, during the middle period and also close together; in leucine (maintenance acid), deaths are late and scattered over a long period. The greater the number of acids in a mixture, the better the average maintenance. When several acids were given, the life spans of many animals were greatly prolonged without metamorphosis. The last animals died when nearly 10 months old.

It is possible to maintain amphibian larvae on isolated amino acids. One acid is insufficient, bi-acid mixtures are rather insufficient. The more complex combinations are sufficient approximately in ratio to the number of acids. However, even many acids are not always sufficient in the concentrations used by us. Differentiation did not advance much beyond the hind limb stage; in some animals, fore-limbs began to appear; in isolated cases indications of beginning tail resorption were noticed.

Diiodo-tyrosine brings on the usual early metamorphosis. Its influence can be modified by growth acids (such as arginine) so that metamorphoses appear later than in diiodo-tyrosine alone.

5506

Bacteriology of Deep Tissue Cultures from Chronic Cervicitis.

HARRY O. MARYAN. (Introduced by F. H. Falls.)

From the Departments of Obstetrics and Gynecology, Bacteriology and Pathology, University of Illinois College of Medicine.

This study of chronic cervicitis deals with the bacteriological examination of organisms in the depths of the ground tissue and also with the histopathological investigation of the sections. Chronic cervicitis is due to a persistent infection in the depths of the compound racemose glands, characterized pathologically by a periglandular round cell infiltration. The cervix, after the primary insult, either from a previous gonorrhoeal infection, the effects of childbirth, or other factors, forms a favorable habitat for the secondary invaders. Menge and Kronig,[1] Arnold and Brody,[2] Barrett, Lash and Pilot,[3] Moench,[4] Pilot and Kantor,[5] and Taylor and Wright[6] are agreed as to the various organisms found on the surface of the pathologic cervical canal; but no mention has been made of the bacterial flora in the depths of the glands.

The cervical tissue was enucleated by Sturmdorf's technique using ether, the electric cautery knife or scalpel. The sterilized enucleated cervix was immediately placed in a small sterile glass jar with a cork stopper and examined within one-half to 2 hours. The culture media was dextrose infusion broth 2%, Bacto dehydrated medium veal infusion pH 7.3, blood agar (human blood 10%), plain agar and egg yolk agar plates. At times 20% ascitic fluid was added to the veal infusion and to the blood agar plates. Ten cc. of veal infusion was used mainly, in test tubes, for inoculation from the surface, and small flasks containing 100 cc. for the ground tissue. Cultured aerobically and anaerobically at 37.5°C., 24 to 48 hours or longer. Smears from cultures were stained by Gram's method. The pyrogallic acid potassium hydrox method in a desiccator was used for anaerobiosis. The terminal hydrogen ion concentration was determined between 5 or 10 days' incubation.

Sugar fermentation reactions of 1% using brom cresol purple

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[1] Menge and Kronig, *Bakteriologic des Weblichen Genitalkanales*, 1897, 1.

[2] Arnold and Brody, *J. Lab. and Clin. Med.*, 1925, **10**, 517.

[3] Barrett, Lash and Pilot, *Am. J. Obst. and Gynec.*, 1925, **9**, 797.

[4] Moench, L., *J. Lab. and Clin. Med.*, 1924, **9**, 289.

[5] Kantor and Pilot, *Surg. Gynec. and Obst.*, 1924, **7**, 96.

[6] Taylor and Wright, *J. Obst. and Gynec. Brit. Emp.*, 1930, **37**, 213.

TABLE I.—SUGAR FERMENTATIONS.

Strains	Dextrose	Lactose	Maltose	Litmus milk Coagulated	Mannite	Salicin	Nitrate	Glycerin	Gelatin	Arabinose	Saccharose	Raffinose	Inulin	Dulcite	
34	+	+	+	+	—	+	+	+	+	—	+	—	—	—	
42	+	+	+	+	+	+	—	+	+	—	+	—	—	—	
44	+	+	+	+	+	—	—	—	+	—	—	+	—	—	
41	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
47	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
39	+	+	+	+	—	—	+	+	—	—	+	—	—	—	Hemolytic
51	—	—	+	+	—	—	—	—	+	—	+	+	—	—	
57	+	—	+	+	+	—	+	—	+	—	+	+	—	—	
43	—	—	—	—	—	+	—	—	—	+	+	+	—	—	

indicator, were tested by utilizing Gordon's,[7] Andrewes and Horder's,[8] Holman's,[9] and Dible's[10] sugar fermentation classifications.

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[7] Gordon, M. H., *Lancet*, 1905, **2**, 1400.

[8] Andrewes and Horder, *Lancet*, 1906, **2**, 708.

[9] Holman, *J. Med. Res.*, 1916, **34**, 388.

[10] Dible, J. H., *J. Path. and Bact.*, 1921, **34**, 3.

Pathogenicity was determined by injecting mice with ¼ cc., ½ cc. and 1 cc. of a 24-hour veal infusion culture. The sediment was observed in 100 cc. plain broth in a small flask. The tissue was ground under aseptic conditions described by Maryan.[11] The outer surface of the tissue was sterilized by searing it through the flame before grinding but thereafter the tissue was sterilized by holding it in boiling water for 15 seconds.

Experiments. From 51 cases, 41 strains of gram positive cocci were isolated and in the remaining 10 cases the cultures were sterile. These organisms were gram positive cocci and are facultative aerobes. Morphologically they occur predominately as lanceolate or oval diplococci surrounded by a halo and in short chains of 3 to 4 cocci but also associated with tetrads and small clusters. They appear pleomorphic. (We have also isolated a number of long chained cocci.) The cocci assume various forms and positions and appear swollen. They have no capsule.

Upon blood agar plates they appear as small moderately elevated pin point to pin head moist grayish white colonies, which grow luxuriantly with no discoloration. Four strains showed large whitish colonies with definite hemolysis, probably hemolytic enterococcus.[12] Upon plain agar they grow similarly with no difficulty. The colonies vary in size from 0.3 mm. to 0.7 mm. in diameter. Upon trans-illuminated light, they appear oval, granular, semi-opaque, and surrounded by a narrow clear zone, just within the outside margin of the colony. The sediment in a test tube of veal infusion appears granular and in streaks radiating downward from the surface. The sediment in a 100 cc. flask of plain broth appears white, stringy and mucoid. These cultures have been kept alive for about 4 months at 37.5°C. They are saccharolytic. From our study of the carbohydrate fermentation, our strains can be classified into a biologically active group, a biologically inactive group, and the variables. They are heat resistant withstanding 60°C. for one-half hour. Out of a series of 72 mice, 12 died, and the same organisms were recovered from the heart in all. The terminal hydrogen ion concentration, by the Hellige method, ranged from pH 4.3 to pH 6.7.

The organisms described correspond morphologically and biologically with the *Streptococcus fecalis* or enterococcus of Dible[11] or the enterococcus described by Arnold.[2] Therefore we call it enterococcus.

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[11] Maryan, H. O., *J. Lab. Clin. Med.*, 1930, **16**, 64.

[12] Weatherall and Dible, *J. Path. and Bact.*, 1929, **4**, 413.

The findings of the histopathological sections will be reported at a future date.

5507

A Simple Continuous Standard for Colorimetric Determination of pH.

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From the Division of Medicine, Stanford University Medical School.

Comparison of an unknown solution containing indicator with standards obtained by superimposing the extreme acid and alkaline colors of the indicator in varying proportions is used in several methods of pH determination. In the simplest of these the standards consist of a series of pairs of test tubes, one tube of each pair containing acid indicator and its mate alkaline indicator.[1] By partitioning a fixed quantity of indicator in different proportions in each pair of tubes a series of color standards is obtained whose pH values have been determined.

The mid-point of such a series is represented by a pair of tubes containing equal concentrations of the indicator. For our purposes they must also contain equal volumes. Starting with such a pair of tubes, if we add to the acid tube from a burette any volume of a solution of the indicator having a concentration twice that in the tube, and from another burette add to the alkaline tube an equal volume of water, the combined color of the tubes may be made to pass continuously through that portion of the indicator range which lies on the acid side of its mid-point. Similarly by adding indicator to the alkaline tube and water to the acid tube the other half of the range may be covered. The original mid-point pair of tubes may be titrated in this manner either to match a given unknown solution or to a color representing any desired pH. The amount of shift of the pH from the mid-point will be equal to log $\frac{A+X}{A}$, when A is one-half of the initial volume in the mid-point tubes, and X is the burette reading. Tables or graphs giving corresponding values of pH difference and burette reading may be constructed. It is apparent that the smaller the initial volumes in the mid-point tubes the more sensitive the end-point will be in the marginal portions of the indicator range.

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[1] Gillespie, L. J., *Soil Science*, 1920, **9**, 115. Clark, W. Mansfield, The Determination of Hydrogen Ions, Edition 1928.

5508

Influence of Repeated Contractions of Muscle on its Lipid Content.

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From the State Institute for the Study of Malignant Disease, Buffalo.

Whereas muscular activity is accompanied by striking changes in carbohydrate content, most attempts to demonstrate changes in the lipid content of muscle have failed. Leathes[1] found no difference in the amount of fat in corresponding muscles of the 2 legs of frogs, after one leg had been stimulated for several hours by a tetanizing current. Winfield[2] obtained equally negative results on excised frog muscle. Since the respiratory quotient of isolated, glycogen-rich amphibian muscle is generally close to unity, indicating a predominant use of carbohydrate, there was the possibility that utilization of fat occurred only in a muscle with low glycogen content. In support of this idea may be cited the observation of Niernierko[3] that stimulation of the isolated gastrocnemius of frogs in winter had no effect on fat content, while in spring, when the glycogen content is known to be low, stimulation resulted in a decrease in fat content of 12 to 31%.

After a fasting period of 24 hours close to 90% of the heat production of rats is derived from fat oxidation, as indicated by a non-protein R. Q. of 0.71. Obviously the muscles of such animals are using chiefly fat (either directly or indirectly) and it therefore seemed worth while to investigate whether the marked increase in oxygen consumption brought about by activity, would result in a demonstrable change in lipid content.

The gastrocnemius of the rat (weighing 1 to 1.5 gm.) was thoroughly ground with sand, transferred to a 100 cc. volumetric flask and boiled for 15 minutes with 75 cc. of a 3 to 1 alcohol-ether mixture on a water bath. After making up to the mark, aliquot portions of the filtered extract were analyzed for free and total cholesterol, using Okey's[4] digitonide method and for phospholipids and total fatty acids, following Bloor's[5] procedure. Oxidation was carried out with standard dichromate-sulfuric acid mixture as recommended by Bloor.[5] The methods were tried out on known mixtures of cholesterol, lecithin and fatty acids before being used for the experiments.

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[1] Leathes, J. P., Problems of Animal Metabolism, London, 1906.

[2] Winfield, G., *J. Physiol.*, 1914, **49**, 171.

[3] Niernierko, W., *Acta Biol. Exp.*, 1929, **3**, 143.

[4] Okey, R., *J. Biol. Chem.*, 1930, **88**, 367.

[5] Bloor, W. R., *J. Biol. Chem.*, 1928, **77**, 53; 1929, **82**, 273.

First, 2 control experiments were performed in which one gastrocnemius was removed from a rat immediately after the induction of amytal anesthesia and the second 1 hour later. The cholesterol and phospholipid content of the muscles of the 2 sides showed good agreement, whereas in one case there was an appreciable difference in the total fatty acid content of the right and left side (Table I).

TABLE I.
Stimulation of rat gastrocnemius.
All values are given in gm. per 100 gm. fresh muscle.

Free cholesterol	Total cholesterol	Phospholipids	Total fatty acids	Free cholesterol	Total cholesterol	Phospholipids	Total fatty acids	Remarks
Right				Left				
0.148	0.159	1.40	2.67	0.137	0.151	1.35	2.69	No stimulation
0.143	0.146	1.56	2.42	0.133	0.136	1.52	2.09	,, ,,
Resting				Stimulated				
0.157	0.167	1.12	2.12	0.136	0.151	1.10	2.05	36 times per min. 1 hr
0.164	0.165	1.13	2.83	0.162	0.165	0.99	2.11	68 ,, ,, ,, 1 ,,
0.126	0.127	1.25	2.51	0.100	0.125	1.18	2.48	68 ,, ,, ,, 2 ,, load 10 gm.
0.122	0.125	1.36	2.88	0.119	0.124	1.45	3.08	68 times per min. 2 hrs load 50 gm.
0.142	0.146	1.21	2.58	0.129	0.141	1.18	2.43	Average of last 4 exp.

In 4 experiments the cut sciatic nerve on one side (after severing branches to muscles other than the gastrocnemius) was stimulated with single induction shocks, the rate being regulated by a metronome. Though in 2 cases the muscle was made to lift a load, signs of fatigue did not set in during the 2 hours of stimulation. The unstimulated gastrocnemius of the other side (with its nerve cut at the start of the experiment) was removed at the same time that the stimulated muscle was taken for analysis. In 3 experiments there was no significant difference in the lipid fractions of the 2 sides. Experiment 2 cannot be regarded as indicating a disappearance of fatty acids during work, since a similar difference in total fatty acids was encountered in one control experiment. Since even the large amount of work performed in the last experiment (8160 twitches with a load of 50 gm.) had failed to cause a diminution in the lipid content, it seemed unprofitable to continue this type of experiment.

Frogs which had remained in the laboratory during the winter and spring and were in a poor nutritional condition, were anesthetized with urethane and the muscles of one leg were fatigued by repeated stimulation of the sciatic with single induced shocks, while the muscles of the resting legs served as control. A decrease in the total fatty acid content of the fatigued muscle was observed in each of the 6 experiments recorded in Table II. Since the water content of the stimulated muscle was found to be higher than that of resting muscle, the average values were calculated in terms of dry weight. In 4 control experiments in which the muscles of the right and left side were compared without previous stimulation, the average values of the 2 sides showed good agreement.

TABLE II.

Stimulation of hind legs of frogs.

Values are given in gm. per 100 gm. fresh muscle. Experiments were made in July at a room temperature of about 25°C.

Total cholesterol	Phospholipids	Total fatty acids	Total cholesterol	Phospholipids	Total fatty acids	Remarks
	Right			Left		
0.061	0.38	0.73	0.062	0.35	0.76	No stimulation
0.069	0.31	0.65	0.060	0.31	0.72	” ”
0.083	0.41	0.77	0.090	0.41	0.67	” ”
0.106	0.51	0.68	0.10	0.45	0.67	” ”
0.079	0.40	0.71	0.078	0.38	0.70	*Av. water content:* R 88.2; L 88.2%
0.67	3.39	6.01	0.66	3.22	5.94	Av. per 100 gm. dry weight
	Resting			Stimulated		
0.046	0.33	0.73	0.045	0.24	0.64	30 times per min. 3 hr.
0.036	0.30	0.75	0.039	0.18	0.52	30 ” ” ” 2 ”
0.050	0.39	0.61	0.043	0.29	0.51	22 ” ” ” 2 ”
0.045	0.39	0.85	0.049	0.28	0.52	30 ” ” ” 2 ”
0.047	0.47	0.79	0.040	0.45	0.51	30 ” ” ” 2 ”
0.059	0.27	0.77	0.049	0.27	0.53	30 ” ” ” 2 ”
0.047	0.36	0.75	0.044	0.28	0.54	*Av. water content:* R 86.8; St. 88.2%
0.356	2.72	5.69	0.373	2.37	4.57	Av. per 100 gm. dry weight

Cuthbertson[6] fatigued muscles of decerebrate cats by faradic stimulation but was unable to demonstrate a decrease in fat content. Similar experiments on rats recorded in this paper were also negative. The failure of contracting mammalian muscle to use its own fat, especially when (as in the present experiments) the respiratory quotient of the whole animal indicates predominantly fat combustion, makes it probable that the muscle derives its energy from blood fat. Determinations of the fat content of arterial and venous blood of muscle should demonstrate this, and Lafon[7] reported that the arteriovenous difference in fat content increases during activity of the muscles lifting the upper lip of the horse. The results obtained on frogs in summer indicate that muscle may use its own fat during prolonged activity. Conditions favoring this utilization seem to be a low glycogen content of muscle and possibly an insufficient supply of blood fat.

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[6] Cuthbertson, D. P., *Biochem. J.*, 1925, **19**, 896.

Summary. The cholesterol, phospholipid and total fatty acid content of the rat gastrocnemius remained unchanged during prolonged and severe muscular work. In frogs in summer the fatigued muscles of one leg contained on an average 19.7% (per dry weight) less fatty acids than the resting muscles of the other leg.

5509

Failure of Nicotine to Alter the Estrus Cycle in the White Rat.

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Ten white female rats of the Wistar strain were injected twice daily with nicotine hydrochloride in a dose of 2 to 3 mg. per kg. in 1 to 1,000 solution. This amount was sufficient to produce convulsions of varying degree, usually becoming less as the experiment progressed. Injections were begun at the age of 2 to 4 weeks, and an equal number of litter mates were injected with equal volumes of 0.85% NaCl solution as controls. Beginning at the age of 3 months in one set of 7 nicotine and 7 control rats, and at 5 months in a set of 3 nicotine and 3 control rats, vaginal smears were examined daily, between 4:30 P. M. and 6:00 P. M., for a period of approximately 3 months. During the course of the experiment, one of the control group was accidentally killed, and another became pregnant, and could not supply data for the test for the period of pregnancy, and until estrus began again after taking away the young. These accidents account for the discrepancy between the total rat days of nicotine and control groups. Comparison of the 2 groups of rats was made by 2 methods, as follows:

Method I. A value of 2 was assigned to each day of complete cornification of the vaginal epithelium, as indicated by the smear, and a value of 1 to each day upon which the smears contained nucleated epithelium (non-cornified) but no leucocytes. The total value for estrus days for the nicotine group was 659 and for the controls, 635. The total rat days for the nicotine group was 1242, and for the controls, 1118. By dividing the value of the estrus days by the total rat days, the "estrus index" may be obtained. For the nicotine rats, this index was $659 \div 1242 = 0.53$; for the controls, $635 \div 1118 = 0.57$.

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[7] Lafon, G., *Compt. rend. Acad. Sc.*, 1913, **156**, 1248.

Method II. The second method of evaluation considered only the days of cornification of vaginal epithelium, each day of cornification being given a value of 1. There were 250 rat days of cornification among the nicotine group, and 228 among the controls. The "cornification index" for the nicotine group was $250 \div 1242 = 0.202$; for the control group, $228 \div 1118 = 0.204$.

Thus by each method of comparison it was shown that nicotine had no effect on the estrus cycle of the white female rat.

5510

Fragility of Human Erythrocytes to Certain Salts.*

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From the Department of Pathology, College of Medicine, Tulane University.

Hober[1] determined that the potassium ion had a greater hemolytic effect than the sodium ion by placing red cells in tubes of hypotonic solutions of these salts and noting the time of appearance of hemolysis in the supernatant fluid. Hamburger[2] proved that the sodium and potassium cations could pass in and out of red cells. Ashby[3] incubated mammalian bloods of different species and determined that blood in which the red cells contained excess of potassium showed greater susceptibility to hemolysis by potassium than by sodium salts, and blood in which the red cells contained excess of sodium showed greater susceptibility to hemolysis by sodium than by potassium salts, and attributed these effects to action on the cell

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* Aided by grant from the David Trautman Schwartz Research Fund.

† I wish to thank the Touro Infirmary for the blood specimens which made this work possible.

[1] Hober, R., *Biochem. Z.*, 1908, **14**, 209.

[2] Hamburger, H. J., *Wiener med. Wochschr.*, 1916, **66**, 521, 575.

[3] Ashby, W., *Am. J. Physiol.*, 1924, **68**, 239, 285.

membrane and colloids of the cell rather than to osmosis. He also showed that the resistance of red cells was increased by standing, by injury of the cells, by increase of the fluid whether isotonic saline or serum, etc., and by carbon dioxide.

Hober and Ashby allowed the solutions to stand until hemolysis occurred. Just how valuable such a procedure might be with reference to the red cell *in vivo* is questioned by the author. There are surely physico-chemical changes in the cell on standing and this changed cell can hardly be compared with the red cell in the living body from a physiological point of view. The author has, therefore, added the blood cells collected by syringe from the patient directly to tubes containing varying concentrations of the salts; sodium chloride, potassium chloride, calcium chloride and magnesium chloride, and has observed the changes. Since the change 5 to 10 minutes after the addition closely corresponded to that of the same solutions 2 hours afterwards when the readings were accomplished with greater ease, the 2 hour readings were accepted as the results. The readings were made at room temperature. The salts were dissolved in distilled water which had a pH of 5.2. The pH of the sodium and potassium chloride solutions remained the same as that of the distilled water but the pH of calcium chloride solutions in the case of the results quoted was 6.2 and that of the magnesium chloride solutions 6.6 to 6.5. A drop of blood was added to the solutions and the reading was made of the concentration at which hemolysis first occurred, approximately 50 specimens being examined.

Hemolysis began in the case of sodium chloride in the 0.40 to 0.45% concentration (44 to 50% isotonic) ; in the case of potassium chloride in the 0.60 to 0.65% concentration (50 to 54% isotonic) ; in the case of magnesium chloride in the 1.00 to 1.05% concentration (52 to 55% isotonic) and in the case of calcium chloride in the 0.80 to 0.90% concentration (54 to 62% isotonic). The differences in the tonicity shown in these results would seem to substantiate the conclusions of Ashby and others[4] since greater resistance to hemolysis is shown by hypotonic salt solutions of sodium chloride than potassium chloride, greater resistance by potassium chloride than magnesium chloride and greater resistance by magnesium chloride than calcium chloride. However, the differences are not great and it would be extremely difficult to conclude that the phenomenon was anything but one of osmosis.

As Ashby has shown, increased resistance of the cells was demonstrated in washed and unwashed cells allowed to stand, whether in the clot, in Ringers, in citrate or in normal saline. The author noted, however, that the range from beginning to complete hemolysis was much less in the case of potassium than the other salts. The results with the calcium and magnesium salts were less constant and less reliable than those with the potassium and sodium salts and their readings were less definite in every case in which they were used.

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[4] Simon, A., *Biochem. Z.*, 1929, **170**, 244.

It was attempted to note any change in fragility of red cells when they were incubated in the salt solutions containing 0.0006 molarity of methylene blue under the assumption that the methylene blue would catalyse the absorption of oxygen by the red cells. No change was noted in the methylene blue solutions from that occurring in solutions of the same concentration of salt but containing no methylene blue. The methylene blue solutions were easily read despite the color change.

5511

Influence of Dextrose and of Low Temperatures on Preservation, Transportation and Viability of Malaria Parasites.

F. M. JOHNS.

From the Laboratory of Clinical Medicine, Tulane School of Medicine.

In a study of the factors underlying continued growth and reproduction of malaria parasites *(Plasmodium vivax)* in blood cells removed from the human host, Bass and Johns[1] found that the addition of 0.5% of dextrose to human blood before defibrination apparently prevented an endosmosis into the parasitized corpuscles of toxic substances present in normal serum that quickly destroyed the parasites at body or other temperatures. That by the addition of dextrose the parasites continued their asexual reproduction at a rate directly dependent upon the temperature, up to a maximum of 40°C.

Confronted with the necessity of preserving and transporting blood containing tertian malaria parasites, I have extended these observations on dextrose content and temperature most conducive to prolonging the viability and consequent infectivity of such parasites.

Blood collected from patients presenting the symptoms of therapeutic "inoculation" malaria was first studied daily in stained preparations of defibrinated blood containing 0.5% to 2% of previously added dextrose, and kept at temperatures of —5 to +15°C. Two such experiments conclusively revealed the longest survival of parasites, as shown by normally stained chromatin and protoplasm, in bloods containing 1% of dextrose and when kept at exactly 0°C. In both instances apparently viable parasites could be demonstrated with ease until the tenth day. Dead and dying parasites, as shown by their staining reactions, were most numerous in the higher temperatures with the passage of each 24 hour period. At —5°C. the parasitized cells began to disintegrate within a few hours. All of the parasites in these experiments were schizonts.

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[1] Bass, C. C., and Johns, F. M., *J. Exp. Med.*, 1912, **16**, 567.

The routine method adopted was as follows: Blood was withdrawn from a patient (presenting tertian malaria parasites) with a dry-sterilized all-glass syringe and immediately run into sterile test tubes marked for 10 cc. and containing 0.2 cc. of a 50% sterile solution of dextrose. Defibrination was accomplished by stirring for 10 minutes with a roughened glass rod. The blood was then transferred to small mouth, rubber-stoppered vaccine bottles of 5 cc. capacity and stored in crushed ice. Transportation over long distances has been accomplished by packing in thermos bottles filled with crushed ice plus ice water.

During the past 4 years I have records of 91 inoculations of blood removed from the patient more than 24 hours. These may be summarized as follows:

TABLE I.

0°C.	No. of inoculations	Infection	Non-infection
1- 4 days	67	58	9
5- 8 ''	11	7	4
9-12 ''	5	1	4
13-15 ''	3	1	2
16-18 '' *	2	1	1
18-20 '' *	3	0	3

* In none of these 5 instances could well stained parasites be demonstrated in stained smears.

Infections have been obtained with bloods shipped by air mail from New Orleans to Los Angeles, or to Jacksonville, Florida; also by combined air mail and train to Denver via Chicago and Billings, Montana. In this latter instance there was an elapsed time of over 5 days.

Inoculations were mostly made on institutionalized patients not subject to probable natural infection.

While control figures are not available on the number of natural immunes present in this community, my impression is that the infectivity of such preserved blood up to 8 days is fully equal to direct transfer of blood from patient to patient.

I wish to thank Dr. H. R. Unsworth, Psychiatrist of the DePaul Sanitarium of New Orleans, for his co-operation by making available the majority of the patients used.

5512

Photodynamic Action of Certain Dyes on the Inactivation of Staphylococcus Bacteriophage.

C. E. CLIFTON. (Introduced by E. W. Schultz.)

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In previous notes the inactivation of staphylococcus bacteriophage by methylene blue (Schultz and Krueger[1]) and by toluidine blue (Clifton and Lawler[2]) was reported.

Various samples of methylene blue in concentrations of 0.1 and 0.01% were mixed with antistaphylococcus bacteriophage, exposed to sunlight for different intervals of time, incubated for 24 hours at 37°C. and then tested for lytic activity. No inactivation was noted when the dye and 'phage were mixed, incubated, and tested in the dark. However, an exposure of 5 minutes, or even less than this time, to indirect sunlight before incubation resulted in complete inactivation of the bacteriophage.

When the methylene blue was added to the bacteriophage suspension in an atmosphere of nitrogen, or in a tube from which the air had been evacuated, no inactivation was noted. This was also true after 30 minutes exposure to direct sunlight and subsequent incubation of these oxygen-free systems. The leucobase of methylene blue likewise did not inactivate this bacteriophage.

Several indophenols having a higher oxidation potential than methylene blue were also tested. They are not photosensitive, did not inactivate the bacteriophage, and were reduced by other oxidizable substances present in the broth.

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[1] Schultz, E. W., and Krueger, A. P., PROC. SOC. EXP. BIOL. AND MED., 1928, **26**, 101.

[2] Clifton, C. E., and Lawler, T. G., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 1041.

Baumberger, Bigotti and Bardwell[3] have likewise reported inhibition in the coagulation of plasma as a result of the photodynamic action of methylene blue and similar photodynamic actions have been reported for various dyes on different systems. In this note evidence is presented that this inactivation is due to a photodynamic action of the dye on the oxidation of the bacteriophage.

Similar results were obtained with toluidine blue although the results are not as strictly reproducible as with methylene blue.

The workers referred to above also observed that certain readily oxidizable substances such as resorcin, casein and tyrosine, will protect the clotting system of plasma against the photodynamic action of methylene blue and of eosin. Zinsser and Tang[4] and Long and Olitsky[5] have also reported that traces of cysteine favor the survival of certain viruses by tending to maintain anaerobic conditions in the suspension.

In our studies cysteine hydrochloride was added to the 'phage suspension to give a concentration of 0.01% and the mixture was then treated with methylene blue (0.01%). No inactivation of the 'phage was noted even after 30 minutes of exposure to sunlight, followed by 24 hours' incubation at 37°C. The controls were completely inactivated.

Tyrosine, even in higher concentrations, did not act as a protective agent.

These results, therefore, indicate that the inactivation of staphylococcus bacteriophage by methylene blue is due to an oxidation of this agent by photosensitized methylene blue in the presence of oxygen.

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[3] Baumberger, J. P., Bigotti, R. T., and Bardwell, K., *Am. J. Physiol.*, 1929, **90**, 277.

[4] Zinsser, H., and Tang, F., *J. Immunol.*, 1929, **17**, 343.

[5] Long, P. H., and Olitsky, P. K., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 380.

5513

II. Effect of Thyroidectomy and Suprarenalectomy on Muscle Tissue Respiration.

J. E. DAVIS AND A. B. HASTINGS.

From the Lasker Foundation for Medical Research and the Department of Medicine, University of Chicago.

In a previous paper[1] it was shown that suprarenalectomy increased the respiration of excised abdominal muscle of mice. This increase was shown to obtain only from about the fifty-fifth to the seventieth day after suprarenalectomy. Further experimentation has shown that this period probably begins nearer the fiftieth day so that in this paper comparisons are made for a period extending from about the fiftieth to the seventieth day after suprarenalectomy. To determine the rôle of the thyroids in this increased respiration a series of mice was first thyroidectomized and then about 3 weeks later suprarenalectomized. The operated mice were kept on a bread and buttermilk diet about 6 weeks, 3 weeks before and 3 weeks after thyroidectomy. After this period the diet was changed to dry bread and grains, the buttermilk being retained as the only drink allowed. The control litter mates received similar treatment. After thyroidectomy the operated mice received subcutaneous injections of one-tenth of a milligram of thyroxin* solution 3 times weekly. The respiration of the excised abdominal muscle of both normal and operated mice (50 to 70 days after suprarenalectomy) was measured using the same technique as reported previously, namely, a modification of the Warburg differential method.

The respiration values obtained in 13 such experiments are shown in Table I which is self-explanatory and similar to Table I of the previous paper.[1] It will be remembered that the last 4 columns express the quantity of lactic acid produced both aerobically and anaerobically in accordance with the Warburg method in terms of cubic millimeters of carbon dioxide produced during the first hour of respiration per milligram of dried tissue.

The 13 experiments represented in this table show in most cases little difference between the respiration values of the normal and of the operated mice. This table shows that the difference of the means of the oxygen consumption values of the 2 groups is only 0.21 with

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[1] Davis, J. E., and Hastings, A. B., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 378.

* Natural thyroxin from British Drug House, London, England.

TABLE I.

A Comparison Between the Respiration of Excised Abdominal Muscle of 13 Pairs of Normal and of Operated Mice Having Thyroids and Suprarenals Removed.

No. of Exp.	Days after suprarenalectomy	Cu. mm. of O_2 consumed per mg. of dried tissue per hr.		Cu. mm. of Extra CO_2 produced aerobically per mg. per hr.		Cu. mm. of CO_2 produced anaerobically per mg. per hr.	
		Normal	Operated	Normal	Operated	Normal	Operated
1	50	6.10	6.68	3.28	3.40	7.89	9.68
2	53	5.82	5.85	3.58	3.63	5.90	5.65
3	54	5.80	5.75	3.04	3.24	7.38	7.40
4	54	5.80	5.75	3.04	3.24	7.38	5.60
5	56	5.03	4.95	2.87	2.74	4.72	7.68
6	58	5.90	4.60	3.46	3.86	6.97	8.94
7	60	5.32	4.85	3.22	3.38	6.00	7.51
8	62	5.57	5.78	3.44	2.87	7.42	7.00
9	63	6.49	6.40	3.21	3.45	5.35	9.53
10	66	5.57	5.31	3.44	2.21	7.42	8.42
11	68	6.59	6.79	3.78	3.96	8.26	9.94
12	70	6.41	6.11	3.24	3.16	5.90	8.26
13	71	6.37	5.20	3.43	3.25	6.05	5.78
Means		5.90	5.69	3.31	3.26	6.66	7.80
Difference of means		0.21		0.05		1.14	
Probable error of difference of means		0.17		0.10		0.34	

a probable error of the same magnitude, whereas it was previously found that, when suprarenalectomy was not preceded by thyroidectomy, the difference was 1.76, which was 9 times the probable error. Likewise, this table shows that the difference of the means of the values of the extra carbon dioxide produced aerobically is only 0.05 with a probable error even greater, whereas it was previously found that, when suprarenalectomy was not preceded by thyroidectomy the difference was 0.59, which was 4 times the probable error. This table also shows that the difference of the means of the values of the carbon dioxide produced anaerobically is 1.14 which is 3 times the probable error, and may possibly be of significance. It was previously found, however, that this difference rose to 2.55 which was 9 times the probable error when suprarenalectomy was not preceded by thyroidectomy. Thus, it is to be noted that suprarenalectomy produced an increase of both aerobic and anaerobic metabolism, an increase which was considerably diminished or even destroyed when suprarenalectomy was preceded by thyroidectomy.

5514

Composition of Human Fat.

AVA JOSEPHINE MC AMIS* AND WILLIAM E. ANDERSON.
(Introduced by L. B. Mendel.)
From the Laboratory of Physiological Chemistry, Yale University.

Inasmuch as there are comparatively few records of analyses of human fat, it seemed desirable to publish the following data on the fat, obtained at necropsy, from the abdominal wall of a rather obese 3-year-old female child, whose death was thought to be due to pituitary disturbance.

The fatty tissue† upon removal from the body was kept under 95% alcohol in an ice box, until an extraction of the fat was made by means of hot alcohol and ether. The pale yellow fat yielded an iodine number of 60.8 (Hanus method). The unsaponifiable material was determined by the modified Kerr-Sorber method.[1] A separation of the fatty acids into saturated and unsaturated fractions was accomplished by means of the lead salt-ether method[2]; and corrections were made for the small quantity of unsaturated fatty acids that contaminated the saturated fraction. No quantitative separation of the component fatty acids was made, but upon brominating a sample of the unsaturated fatty acids, a slight precipitate insoluble in ether formed, suggesting the presence of fatty acids more unsaturated than linoleic acid.

The analytical data obtained are included in the following table:

	% of fat	
Unsaponifiable matter	0.4	
Fatty Acids	93.0	
Saturated fatty acids	31.0	
Unsaturated fatty acids	60.0	(Iodine Number 102)

These results are in fair agreement with the more extensive analyses reported by Eckstein[3] for the subcutaneous fat obtained at biopsy from an obese adult.

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* Fellow in Medical Sciences, National Research Council, 1929-31.

† This material was made available through the kindness of Dr. I. H. Perry of the Department of Pathology.

[1] Hertwig, R., Jamieson, G. S., Baughman, W. F., and Bailey, L. H., *J. Oil and Fat Ind.*, 1926, **3**, 64.

[2] Jamieson, G. S., *J. Assoc. Official Agr. Chem.*, 1928, **11**, 303.

[3] Eckstein, H. C., *J. Biol. Chem.*, 1925, **64**, 797.

5515

Anorexia Characteristic of Lack of the Vitamin B Complex: The Rôles of the Individual Components.

ETHEL BURACK[1] AND GEORGE R. COWGILL.[2]

From the Laboratory of Physiological Chemistry, Yale University.

Fifteen dogs subsisted on an artificial food mixture consisting of the highly-purified Casein-Harris,[3] sucrose, butter fat, lard, salt mixture and bone ash. The amounts of these ingredients were calculated according to the scheme described elsewhere.[4] This diet is much more highly purified with respect to the water-soluble vitamin factors than any other ration hitherto employed in this laboratory for feeding experiments with dogs. When anorexia supervened, the following materials were tested for their potency in restoring the urge to eat: (1) a fuller's earth adsorbate[5] containing the antineuritic vitamin B relatively free from the heat-stable G factor; (2) autoclaved yeast, devoid of the antineuritic substance (pigeon tests) but containing the antipellagra vitamin G (tests with young rats); (3) a concentrate of water-soluble vitamins present in milk[6]; (4) combinations of the adsorbate with the autoclaved yeast and milk vitamin concentrate respectively; (5) whole yeast[7]; (6) yeast vitamin powder (Harris) lot 1089[3]; (7)tikitiki[8]; (8) juice of canned tomatoes; and (9) vitavose.[9]

One hundred ninety-nine trials were made; their distribution among test substances is shown in the accompanying table.

It will be noticed that only 26% of the adsorbate tests were effective. This may be explained in two ways: either (1) the antineuritic vitamin is the sole effective agent, but there is variable elution of the

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[1] Alpha Xi Delta Fellow of the American Association of University Women, 1930-1931.

[2] The expenses of this research were defrayed in part by grants from the Committee on Therapeutic Research, Council on Pharmacy and Chemistry, American Medical Association, and from the Research Fund at Yale University School of Medicine.

[3] From the Harris Laboratories, Tuckahoe, New York.

[4] Cowgill, George R., *J. Biol. Chem.*, 1923, **56**, 725.

[5] Kindly furnished by Dr. C. N. Frey, Fleischmann Research Laboratories, New York, N. Y.

[6] Kindly supplied by Dr. G. C. Supplee, Dry Milk Company, Bainbridge, N. Y.

[7] From the Northwestern Yeast Company, Chicago, Ill.

[8] An alcoholic extract of rice polishings obtained from the Bureau of Science Manila, P. I.

[9] From E. R. Squibb and Sons, New York, N. Y.

TABLE I.

Substance Tested	Total No. of tests	Effective Tests	
		Number	% of Total
Fuller's earth adsorbate	46	12	26
Tikitiki	10	10	100
Autoclaved yeast	11	0	0
Fuller's earth adsorbate and autoclaved yeast	53	32	60
Whole yeast	36	35	97
Yeast vitamine powder (Harris)	6	6	100
Milk vitamin concentrate	9	7	78
Fuller's earth adsorbate and milk vitamin concentrate	7	7	100
Vitavose	7	7	100
Tomato juice	14	13	93
	199		

vitamin from the fuller's earth in the alimentary tract, or (2) another factor besides the antineuritic substance is required. In support of the latter view, we may offer the following considerations: these cases occurred either within the first month of subsistence on the diet, when tissue stores of the hypothetical agent may not have been exhausted, or when relatively enormous doses of the adsorbate were given, in which case the presence of the unknown factor as an "impurity" in the adsorbate would account for the results.

In view of the excellent results with tikitiki, a material practically devoid of the heat-stable vitamin G,[10] it appears proper to conclude that this dietary factor plays no rôle. This is confirmed by the uniformly negative results obtained with autoclaved yeast.

The failure to get 100% positive tests in the trials with the combination of adsorbate and autoclaved yeast may be due to (1) the trial being made late in the experimental period (after 2 months) when tissue stores of the unknown substance may have been depleted, or (2) assuming autoclaved yeast to contain this substance, when subminimal amounts of such yeast were given.

It will be noticed that in only one case was whole yeast ineffective. This negative instance was without doubt due to administration of a subminimal dose; repetition with a slightly larger dose gave an excellent response. The yeast vitamine powder (Harris), lot 1089, proved efficacious in every trial.

The remaining tests cited in the table may be interpreted as supporting either of the following views: (1) the antineuritic vitamin is the sole etiological agent; or (2) another substance, not vitamin

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[10] Evans, H. M., and Burr, G. O., *J. Biol. Chem.*, 1928, 77, 231.

G, is required to supplement the antineuritic factor in preventing and correcting the anorexia under investigation. In order to reach a decision concerning these possibilities, further experiments involving parenteral administrations of the antineuritic vitamin are planned.

5516

Hemophilia.

CARROLL LA FLEUR BIRCH. (Introduced by C. S. Williamson.)

From the Departments of Medicine and Orthopedics, University of Illinois College of Medicine.

Hemophilia is limited to the male sex while it is transmitted through the unaffected female. Hence she must potentially have the disease. There are two possibilities. Is the disease manifest only in the presence of the male sex organs or are its symptoms held in abeyance in the presence of the female sex organs?

Two high grade hemophiliacs have been treated with ovarian extract. The first, age 10 years, had been under observation for 18 months prior to the institution of treatment. During this time he was admitted to the hospital several times in severe bleeding phases necessitating transfusion. The coagulation time of the blood usually exceeded one hour. Beginning May 5, 1930, he was given ovarian extract daily until January 1, 1931. He has remained symptom-free with normal blood findings to date (March 17, 1931).

This patient's older brother, the more severe hemophiliac of the 2, was placed on ovarian extract April 5, 1930. After his blood findings became normal (May 2, 1930) a fresh human ovarian transplant was made into the anterior abdominal wall. (The idea of the ovarian transplant was suggested by Dr. H. B. Thomas and he performed the operation.) There was no excessive hemorrhage at the time of the operation. This patient remained free from symptoms with normal blood findings until October 8, 1930, (5 months after the transplant) at which time he had a bleeding phase.

The blood platelets in hemophilia are normal in number and normal morphologically. The resistance of the platelets was measured just as the resistance of red blood cells by the fragility test. The 12 test tubes were arranged as for an ordinary fragility test with salt concentrations varying from 0.28% to 0.5%. Normal red cells begin to hemolize at about 0.4% or 0.38%. Normal platelets also hemolize at about this level.

Method: 5 cc. of hemophilic blood is drawn from a vein. It is centrifuged at low speed for 5 minutes to precipitate the red cells. The blood platelets are found in a homogeneous suspension in the blood plasma above the precipitated red cells. Two drops of the platelet suspension are added to each of the 12 tubes and mixed, and allowed to stand for 2 hours. Then a drop from the bottom of each of these tubes is placed on a slide, covered, and examined with oil immersion lens. The beginning of hemolysis of a platelet is shown by its imbibing water and increasing in size, it elongates and becomes gelatinous in appearance. Normal platelets begin to hemolize at about 0.38%. In hemophilia the blood platelets remain intact throughout the entire series. We then elongated the series to include from distilled water to 5% NaCl. The platelets remained entirely whole and normal in contour in distilled water for 12 hours. They also showed no signs of crenation until they reached the 5% saline. In other words the platelets are very resistant to hypo- and hypertonic salt solution.

Could this increase in resistance of the platelets be overcome, and if so would the blood coagulate in normal time? We tried many chemicals without success. Then we thought of mechanical traumatization. Blood was drawn from the vein, centrifuged at low speed to precipitate the red cells. The supernatant fluid consisting of plasma and platelets was pipetted off, placed in mortar and traumatized with a pestle for 2 minutes. This solution of traumatized platelets was then returned to the blood cells from which it was taken and mixed by inverting once. A control coagulation time with undisturbed platelets was kept in each test. In every instance the tube in which the platelets were traumatized coagulated many minutes before the control tube and usually it coagulated in normal time. An average of 10 control coagulation times (without traumatization of the platelets) was 94 minutes; an average of the same 10 with traumatization of the platelets was 12 minutes.

Conclusions. 1. Ovarian extract and transplant controlled the bleeding of 2 high grade hemophiliacs for a period of 11 and 5 months respectively. 2. Blood platelets of hemophiliacs have a greatly increased resistance both to hypo- and hypertonic salt solution. 3. When the resistance of hemophiliac platelets is overcome mechanically the blood coagulates in normal time.

5517

Studies on the Hookworm Larva as a Vector of Pathogenic Micro-Organisms: Ingestion of Bacteria by Hookworm Larvae.

WILLIAM HARVEY PERKINS. (Introduced by J. H. Musser.)

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Hookworm larvae in their early stages of development are intimately associated with materials heavily contaminated with pathogenic microorganisms. The present investigations are directed toward the possibility that these larvae might act as vectors in the dissemination of certain diseases related in their epidemiology to soil contamination.

The first requirement was to establish the nature of the association existing between larvae and micro-organisms. McCoy[1] has shown that hookworm larvae are able to grow to the infective stage on 22 out of 25 species of bacteria tested. He concludes that all of the evidence from his experiments indicates that living bacteria constitute the essential food utilized by hookworm larvae in developing to the infective stage.

The present work was undertaken to determine whether or not these bacteria could be ingested in their intact form. Certain evidence is presented to show that this is a physical possibility. Of less certainty is the demonstration of bacteria within various portions of the alimentary tract of immature larvae and within the sheath spaces of infective larvae.

The observations were made on living larvae by means of a technic in which the larvae may be studied under the oil immersion power of the microscope. The larvae therefore show no alteration or distortion of structure such as might be produced by death, staining or fixing of the specimens. The observations and measurements can readily be made during more or less prolonged periods of inactivity of the larva.

The technic depends upon the imprisonment of an actively swimming larva in a droplet of water surrounded and covered by cedar immersion oil. The larva is removed from the culture by means of a capillary pipette and transferred to a glass slide. The water medium is reduced to a tiny droplet slightly larger in diameter than the length of the larva by drawing off most of the drop with a capillary pipette and then allowing evaporation to complete reduction to the desired size. At the proper instant a large drop of cedar oil is allowed to fall directly over the water droplet. A cover-slip completes the preparation.

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[1] McCoy, Oliver P., *Am. J. Hygiene*, 1929, **10**, 140.

If larvae are suspected of carrying bacteria it is necessary to demonstrate that they are physically capable of ingesting bodies of bacterial magnitude.

For this purpose *Necator americanus* larvae from patients in the Charity Hospital were examined from within a few hours after hatching to the time of their second ecdysis. The parts measured were the diameters at the base of the oral cup, the inlet and greatest width of the esophageal cylinder and the greatest observed distensibility of the canal of the esophagus, bulbus esophagi and intermediate body.

From this series of measurements it was found that in all of the structures named the dimensions were of such size as to accommodate bacteria of at least the size of *Bacillus coli.* It is believed that none of the flexible canals of the esophagus or bulb were seen at their greatest distensibility.

The second portion of the experiments consisted of attempts to visualize bacteria within the bodies of the larvae.

Larvae immediately after hatching were placed in cultures containing heavy suspensions of *Bacillus coli* or *Staphylococcus aureus* or were left in the original filtrate from the Baermann isolating apparatus. At intervals of several minutes, hours or days the larvae were removed and examined.

Three series of larvae were studied:

Series A consisted of 25 larvae which had been living since isolation in suspensions of colon bacilli and staphylococci. All larvae were examined within the first 4 days after hatching. All were active and their mouth parts still open.

Nine of the series (36%) showed motile bodies within the oral cavity, esophageal cylinder or lumen of the intestine. These bodies were round, oval or rod-shaped, smooth surfaced, darkly refractile and could not be visually differentiated from the colon bacilli and staphylococci seen immediately adjacent to them in the surrounding medium.

Series B. Eleven larvae immediately after hatching and isolation were placed in suspensions of *Bacillus coli.* All of the larvae matured in the suspensions and were examined at intervals of 1 to 31 days after reaching the infective sheathed stage. No bacteriform bodies were seen within the alimentary tract of any of this series, but in 7 (63.6%) bodies similar to those seen in Series A were found within the space between the cuticle of the larvae and their sheaths.

Series C. Forty-three larvae were matured in the original filtrate from the Baermann isolator. This filtrate contained colon bacilli and staphylococci as determined by culture. The larvae were examined at intervals up to the sixteenth day after the second ecdysis. Twenty-two (51.1%) presented one or more bacteria-like bodies within their sheath spaces but none within their alimentary tracts. The average number of such particles was 12 per larva.

Of the total of 79 larvae of the 3 series 38 (48.1%) showed the presence of bodies of bacterial morphology and magnitude within some portion of their alimentary tracts or sheath spaces.

It cannot be positively asserted at this time that these bodies are actually bacterial or even viable.

Preliminary experiments have been performed with the purpose of liberating these bodies by amputation of the tail end of the sheath. Results have shown certain evidence that the particles in question are actually bacterial and can be cultured. This will be investigated further.

Conclusions. 1. The mouth parts and alimentary canal of immature larvae of *Necator americanus* are of such size as to accommodate particulate matter of bacterial magnitude. 2. Particles morphologically resembling colon bacilli and staphylococci have been seen within various portions of the alimentary canal of immature larvae of *Necator americanus* which had been living in a medium containing these micro-organisms. 3. Sheathed larvae of *Necator americanus* have been seen to carry bacteriform bodies within their sheath spaces for periods of from 1 to 31 days after the second ecdysis.

5518

Observations on the Nutritive Value of Certain Fats.

CLAIRE E. GRAHAM AND WENDELL H. GRIFFITH.

From the Department of Biological Chemistry, St. Louis University School of Medicine.

In connection with experiments concerned with the growth of rats on low-fat diets, observations have been made which emphasize the nutritive value of certain fats, and illustrate the marked variance in the results obtained in different laboratories with a fat such as cod liver oil. The basal diet introduced by Burr[1] (purified casein —25, commercial sucrose —75, modified Osborne and Mendel salt mixture —4) was used and was supplemented daily with 0.5 gm. of whole dried yeast and 9 drops of cod liver oil unless stated otherwise. On this diet male rats, 4 weeks of age, increased in weight to 200 gm. in approximately 60 days. These rats (Group 1) exhibited feet and tail signs apparently indentical with those noted by Burr and Burr[2, 3] in rats on a fat-free diet. The more serious effects reported by these authors were not evident in our experiments, possibly because we used whole yeast in place of ether-extracted yeast and cod liver oil in place of the non-saponifiable fraction of that oil. The feet and tail signs were not prevented by doubling the daily yeast supplement (Group 7), or by adding an active extract of rice polishings (Group 10) or by adding an active extract of liver (Group 8). The signs were, for the most part, prevented by wheat germ oil (Group 4), lard (Group 6), and whole liver (Group 9). Wheat germ oil was most effective, and lard was least effective, although the effect of the lard was greater when the daily supplement of cod liver oil was decreased (Group 5). The severity of the condition of the tails of the rats given 12 drops of cod liver oil daily was even worse than that of the animals given 9 drops daily. It was evident that the total fat in these diets was secondary in importance to the ratio of cod liver oil to the other fats (Groups 2, 3 and 4).

Changes in the feet and tail of the rat have been reported by other investigators and appear to be associated with various types of nutritive deficiencies.[4, 5, 6, 7] However, the condition in our rats seemed to be due to the same cause as that reported by Burr and Burr, since it was prevented by wheat germ oil, lard and whole liver and was not prevented by yeast or other sources of the vitamins B_1 and B_2. On the other hand, our results differed markedly from theirs in that the signs appeared on a diet which was not free from fat and which contained cod liver oil. Burr and Burr found that 3 drops of cod liver oil daily were very effective in preventing the signs.

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[1] Evans, H. M., and Burr, G. O., PROC. SOC. EXP. BIOL. AND MED., 1928, **25**, 390.

[2] Burr, G. O., and Burr, M. M., *J. Biol. Chem.*, 1929, **82**, 345.

[3] Burr, G. O., and Burr, M. M., *J. Biol. Chem.*, 1930, **86**, 587.

[4] Goldberger, J., and Lillie, R. D., *P. H. R., U. S. P. H. S.*, 1926, **41**, 1025.

[5] Smith, A. H., and Bogin, M., *Am. J. Path.*, 1927, **3**, 67.

[6] Parsons, H. T., *J. Biol. Chem.*, 1931, **90**, 351.

[7] McAmis, A. J., Anderson, W. E., and Mendel, L. B., *J. Biol. Chem.*, 1929, **82**, 247.

TABLE I.

Group	No. of rats showing feet or tail signs after 60 to 80 days on diet.			Daily dietary supplement	
	+	±	—	Yeast gm.	Cod liver oil drops
1	12	1	1	0.5	9
2	10	0	0	0.5	12
3	6	2	2	0.5	9 + 3 drops lard
4	0	2	8	0.5	9 + 3 drops wheat germ oil
5	0	2	8	0.5	3 + 6 drops lard
6	0	1	9	0.5	9 (10% lard in basal diet)
7	9	1	0	1.0	9
8	10	0	0	1.0	9 + 0.5 cc. aqueous liver extract
9	0	3	7	0.5	9 + 0.5 gm. whole dried hog liver
10	10	0	0	0.5	9 + 4 drops extract of rice polishings (tiki tiki)

Recent investigations in several laboratories[3, 8, 9] have emphasized the need for caution in stating the dietary requirements of the growing rat, the experimental animal most commonly used in nutrition experiments. It is possible that failure to recognize the presence of variable amounts of the known dietary factors and also of variable amounts of known and unknown toxic substances in such standard basic food materials as cod liver oil and yeast may be largely responsible for the confusion which exists at present concerning the essential constituents of the diet. Obviously, additional information is required before it may be stated that the feet and tail signs in our experiments were the result of a deficiency in any food factor or the result of a toxic substance present in the cod liver oil used.

The data on the growth, food consumption and water intake of these rats will be reported later.

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[8] Honeywell, H. E., Dutcher, R. A., and Ely, J. O., *J. Nutrition*, 1931, **3**, 491.

[9] Norris, E. R., and Church, A. E., *J. Biol. Chem.*, 1930, **89**, 437.

5519

Toxicity of Synthetic Methanol.

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The importance of synthetic methanol as an industrial solvent is rapidly increasing. Because of the rather widespread belief among the laity that the synthetic product is less toxic than natural methanol this investigation was undertaken. Although the purest synthetic* and the purest natural† methyl alcohol were used it was observed that the former had very much less odor than the latter. Possibly this is the basis for the popular belief that the synthetic product is the less toxic.

Male white mice between 18 and 20 gm. in weight were selected. Two groups of 30 mice in each experiment were injected intraperitoneally with equal volumes of the synthetic or the natural methyl alcohol diluted 50% by volume with water. Three experiments were performed in which 0.4 cc., 0.5 cc., and 0.6 cc. of solution per 25 gm. of mouse were injected. An arbitrary time interval of 72 hours subsequent to the injection was taken as the period of survival. Post mortem examination was performed routinely and in no instance could death be attributed to causes other than methyl alcohol. The table indicates the results.

TABLE I.—*The Toxicity of Synthetic Methanol.*

	No. mice injected	Vol. of 50% methanol solution per 25 gm. weight	% dead in 72 hours
		cc.	
Synthetic	30	0.4	20
Natural	30	0.4	16.7
Synthetic	30	0.5	30
Natural	30	0.5	36.7
Synthetic	30	0.6	90
Natural	30	0.6	96.7

Loss of pupillary reaction to light and failure to respond to visual stimuli ensued in rabbits when either synthetic (3 animals) or natural (3 animals) methanol was administered by stomach tube in a dose of 6 cc. per kilogram of body-weight every 24 hours for 3 days. Each dose is somewhat higher than the single narcotic dose given by Sollmann and Hanzlik,[1] but no narcosis was produced. However, the series was small, consisting of 3 animals in each group. Nevertheless, it seems justifiable to conclude that there is no significant difference in the two products as regards their effects presumably on the optic nerve in the rabbit.

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* Supplied by Commercial Solvents Corporation.

† Supplied by Mallinckrodt Chemical Company.

Conclusions. 1. There is no difference in toxicity between synthetic and natural methanol when injected intraperitoneally into the white mouse. 2. Both the synthetic and natural methanol in equal amounts produce apparent blindness in rabbits.

It appears that the pathology of methyl alcohol poisoning is quite well established. The object of the experiments as submitted was to compare the toxicity of the synthetic product with that of the natural methanol. In acute poisoning with methyl alcohol frequently no gross pathological changes are observed; the characteristic changes in the retina appear only somewhat late and subsequent to optic atrophy.

5520

Determination of Plasma Phosphatase.

AARON BODANSKY.
With the Technical Assistance of Lois Hallman and R. Bonoff.
From the Laboratory Division of the Hospital for Joint Diseases, New York City.

The significance of phosphatase in bone physiology was suggested by Robison and his coworkers. Increased plasma phosphatase was recently reported by Kay in *ostitis fibrosa, ostitis deformans,* active rickets and *osteomalacia.* Kay also reported a method for the determination of plasma phosphatase.[1] In an experimental study of plasma phosphatase in *ostitis fibrosa,* which we produced in young guinea pigs and dogs by prolonged treatment with parathormone[2, 3, 4, 5] we found Kay's method of determination of plasma phosphatase unsatisfactory. We have avoided the disadvantages of Kay's method by the use of an M/10 solution of sodium diethylbarbiturate (veronal) as a buffer.* When it is added to plasma in the proportions which we employ, the resulting mixture has a pH of about 8.6. This reaction is not changed after the addition of the substrate—a solution of sodium beta-glycerophosphate (Boots Drug Company, Nottingham, England) containing about 5 mg. of phosphorus per cc.† ‡ Nor does the pH change perceptibly during the action of the phosphatase, even when continued for 72 hours.

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[1] Sollmann and Hanzlik, Experimental Pharmacology, Philadelphia, 1928, 270.

[1] Kay, H. D., *J. Biol. Chem.*, 1930, **89**, 235, 249. In the latter paper Kay also reviews the previous work of Robison and associates.

[2] Bodansky, A., Blair, J. E., and Jaffe, H. L., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 708; *J. Biol. Chem.*, 1930, **88**, 629.

[3] Jaffe, H. L., Bodansky, A., and Blair, J. E., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 710; *Arch. Pathol.*, 1931, **11**, 207.

Our procedure follows: Two cc. of twice centrifuged plasma are sufficient. To each cc. of plasma are added 7 cc. of water and 2 cc. of the veronal solution. A 10 cc. aliquot containing 1 cc. of plasma is withdrawn into a glass stoppered test tube and 1 cc. of the glycerophosphate solution is added. After a single inversion of the tube it is placed in the water bath at 37°. (No preservative is necessary, the toluene added with the glycerophosphate being sufficient.) After 2 hours the tube is rapidly cooled, and 5 cc. of 10% trichloracetic acid are added. About 14 cc. of filtrate are obtained. The total inorganic phosphate is determined in duplicate or triplicate aliquots by a modification of the Kuttner-Cohen method.[6] The plasma phosphorus is deducted and the phosphatase activity is calculated in terms of units (mg. of inorganic phosphorus liberated in 1 hour) per 100 cc. of plasma.

A control determination of plasma phosphorus may be made upon an aliquot of the plasma-veronal mixture (without glycerophosphate), after keeping it in the water bath for 2 hours. However, this treatment affects the phosphorus values so little (although a slight rise is consistent) that a plasma phosphorus determination on an aliquot not so treated or on a sample of the original plasma is permissible.

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[4] Jaffe, H. L., and Bodansky, A., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 795; *J. Exp. Med.*, 1930, **52**, 669.

[5] Bodansky, A., and Jaffe, H. L., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 797; *J. Exp. Med.*, 1931, **53**, No. 5 (May).

* L. Michaelis recommended the use of veronal as a buffer within the range of pH 6.8 to 9.6 (*J. Biol. Chem.*, 1930, **87**, 33). Levene and Dillon used a veronal-HCl mixture (pH 8.6) to buffer the hydrolysis of nucleic acid by intestinal phosphatase (*J. Biol. Chem.*, 1930, **88**, 753).

† We prefer a fresh solution, although it keeps well when preserved with a drop of toluene in the refrigerator.

‡ We are under obligation to Dr. H. D. Kay for our first supply of sodium beta-glycerophosphate.

[6] Bodansky, A., Hallman, L., and Bonoff, R., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 762.

The advantages of our technique are as follows: (1) The volume of plasma required is small, the details of the test are simple and conveniently carried out. (2) The pH of the plasma-veronal-glycerophosphate mixture remains practically constant at about 8.6. (3) The hydrolysis of the glycerophosphate is about twice as rapid as in Kay's procedure. (4) The reaction is retarded so little during the first 2 hours that the *initial* velocity may be calculated in terms of mg. of phosphorus liberated per cc. per hour without substantial error. (5) The phosphorus liberated by 1 cc. in 2 hours is in all cases ample for several aliquot determinations. (6) Plasma phosphatase tests of the same sample or of samples secured from the same subject 24 hours apart check very well; samples obtained at greater intervals may show differences, probably attributable to physiological variability.

With the above technique as a basis, empirical factors may be determined which allow the use of 0.2 cc. of plasma for a phosphatase determination with an accuracy of ±10% when larger quantities of plasma are not available.

5521

Deviation from the "Beer Law" in the Kuttner-Cohen Method for Determination of Phosphorus.

AARON BODANSKY, LOIS HALLMAN AND RISSEL BONOFF.

From the Laboratory Division of the Hospital for Joint Diseases, New York City.

Recently, when determinations of inorganic phosphate in mixtures containing sodium glycerophosphate (used as a substrate in plasma phosphatase determinations) were required, the use of heat involved in the Benedict-Theis method rendered it obviously unsuitable. The many advantages of the Kuttner-Cohen method,[1] with the modifications later suggested by Kuttner and Lichtenstein[2] and Raymond and Levene,[3] recommended themselves to us. We found this method capable of yielding very accurate results (within ±2% of the known quantities), after correction for the considerable deviations from the "Beer law". These deviations were determined by a large number of analyses, charts were constructed and tables compiled for more convenient reading.

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[1] Kuttner, T., and Cohen, H. R., *J. Biol. Chem.*, 1927, **75**, 517.

[2] Kuttner, T., and Lichtenstein, L., *J. Biol. Chem.*, 1930, **86**, 671.

[3] Raymond, A. L., and Levene, P. A., *J. Biol. Chem.*, 1928, **79**, 628.

We use 1 cc. of plasma when it is expected to contain 5 mg. of phosphorus per 100 cc. or more, and 2 cc. of plasma when it is expected to contain less. Ten cc. of water are added and 5 cc. of 10% trichloracetic acid. About 14 cc. of filtrate are available. Four cc. of the acid-molybdate mixture is added to an aliquot diluted to 5 cc., the tube is mixed by tapping, and 1 cc. of stannous chloride solution* is added. Several standards of different concentrations may be employed in a series of tests.

We found it convenient to employ a single standard containing 0.02 mg. per 5 cc. We usually include in one series about 18 tests, triplicate 0.02 mg. standards, and, as a check upon all foreseen and unforeseen sources of error, several solutions of known concentration covering approximately the same range as the unknowns. After calculation of the results the correction is applied.

When the solution compared with the standard is of a greatly different concentration, the less concentrated solution has a grayish tinge that may introduce some uncertainty into the comparison and for that reason we accept our corrected values only when the aliquot compared against the 0.02 mg. standard contains between .0150 and .0300 mg. of phosphorus. After a test of one aliquot enough filtrate is available for duplicate or triplicate determinations on aliquots chosen in accordance with the results of the first test.

An abbreviated table of corrections for use with a standard containing 0.0200 mg. follows:

TABLE I.

Calculated mg. P.	Correction mg. P.	Calculated mg. P.	Correction mg. P.	Calculated mg. P.	Correction mg. P.
.0100	—.0013	.0220	+.0003	.0340	+.0030
.0120	—.0011	.0240	+.0008	.0360	+.0034
.0140	—.0008	.0260	+.0012	.0380	+.0038
.0160	—.0006	.0280	+.0016	.0400	+.0043
.0180	—.0003	.0300	+.0021	.0420	+.0047
.0200	.0000	.0320	+.0026	.0440	+.0052

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* We prefer the more concentrated solution of stannous chloride used by Raymond and Levene.[3]

5522

Individual Variation in Fasting Blood Sugar.

G. WALTERMANN HOLT AND ESTHER M. GREISHEIMER.

From the Department of Physiology, The University of Minnesota.

The individual variation in fasting blood sugar was investigated in 4 healthy adults (2 women and 2 men), on successive days over a period of 3 weeks. The subjects were permitted to eat their usual diets. After a 12-hour fast, the blood was drawn by venepuncture.

Protein removal was effected, in the freshly drawn sample, by the Somogyi[1] zinc precipitation method. The glucose content of the filtrate was determined by the improved copper-iodometric titration of Shaffer.[2] All determinations were made in duplicate.

The data for each individual and for the group as a whole were analyzed statistically. The means, with the minimal and maximal values, standard deviations, and coefficients of variation, are presented in Table I.

TABLE I.
Variation in fasting blood sugar in mgm. %.

Subject	Days	Mean	Minimum Maximum	Standard Deviation	Coefficient of variation
1.	20	93.37±0.97	73.2—104.4	6.41±0.68	6.87
2.	20	86.18±0.52	80.4— 93.0	3.46±0.37	4.02
3.	21	88.73±0.86	76.7— 97.9	5.84±0.61	6.58
4.	15	84.00±0.56	76.7— 88.6	3.24±0.40	3.86
Group		88.35±0.47	73.2—104.4	6.04±0.33	6.84

The mean value is lower than that found by other methods, since it represents only the true glucose. The mean value found for the group of 42 individuals in the glucose tolerance study[2] was 83.12±0.56 mgm. %.

The minimum and maximum values show a spread of 31.2, 12.6, 21.2, and 11.9 mg., for subjects 1, 2, 3, and 4, respectively. Since no attempt was made to keep the diet uniform for the 4 individuals, nor in the same individual from day to day, such differences were anticipated. The standard deviation for any subject does not differ significantly from that previously found in the earlier group of 42.[2] The latter was 5.38±0.40.

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[1] Somogyi, M., PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 353.

[2] Holt, G. W., and Greisheimer, Esther M., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 547.

The coefficients of variation compare favorably with that in the previous study, which was 6.5.

The authors conclude that the fasting blood sugar in any normal individual varies from day to day as much as it does in a large group of normal individuals taken at random.

Thanks are due Mr. Carroll J. Bellis, Mr. Dean A. Collins, and Miss Minnie C. Sherva, who so willingly acted as volunteers.

5523

Peripheral and Central Chemical Control of Pulmonary Ventilation.

HARRIET OWEN AND ROBERT GESELL.

From the Department of Physiology, University of Michigan.

Until the recent work of Heymans there has been little evidence for reflex chemical control of pulmonary ventilation. The demonstration of increased respiratory movements from perfusion of the carotid sinus with carbonated and deoxygenated blood calls for a study of the relative values of peripheral and central chemical control.[1] Several procedures were used.

The effects of injection of sodium cyanide, sodium sulphide, sodium carbonate and sodium bicarbonate into the carotid arteries after occlusion of the vertebral and external carotid arteries and denervation of one carotid sinus were noted. Injection of sodium cyanide on the side on which the innervation of the carotid sinus was intact invariably produced increased ventilation. Injection on the denervated side produced relatively small or no increase in ventilation followed by depression. In general the effects of intra-arterial injection of sodium sulphide were similar to those produced by cyanide. Painting the innervated sinus with sodium cyanide or sulphide elicited increased ventilation. This response was demonstrated to be due to local action. Intravenous injection of sodium cyanide or sulphide after double vagotomy and double sinus denervation was relatively ineffective or entirely ineffective in augmenting ventilation. Late depression of ventilation was not uncommon. Similar results were obtained if the common carotid arteries and the internal occipital arteries were occluded as a substitute for denervation. Injection of cyanide into the 4th ventricle was found to produce immediate excitation or depression without initial excitation. It was concluded that this action was local. In several experiments in which sodium sulphide was injected into the fourth ventricle, immediate, well sustained and marked augmentation of ventilation invariably occurred.

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[1] Bouckaert, Dautrebande and Heymans, *J. Physiol.*, 1931, **71**, 5.

Sodium carbonate produced marked apnea when injected into the carotid arteries on both the denervated and innervated side. Injection of sodium bicarbonate on either side produced the typical augmentation of ventilation. After double vagotomy and double carotid sinus denervation, intravenous injection of sodium carbonate produced apnea. Administration of carbon dioxide in a 30% oxygen mixture after double vagotomy and occlusion of the carotid sinuses elicited strong stimulation of the respiratory muscles. Exterior flooding of the carotid sinus with bicarbonate or carbonate solutions appeared to have no effect on pulmonary ventilation.

The relatively small increase in ventilation produced by intravenous injection of cyanide and sulphide after vagotomy and double carotid sinus denervation agrees with the small increase in ventilation under similar conditions on administration of nitrogen as noted by Bouckaert, Dautrebande and Heymans.[1]

Our findings that denervation had relatively little effect on the action of sodium carbonate, sodium bicarbonate and carbon dioxide call for special consideration of a dual mechanism of control of ventilation by oxygen lack and carbon dioxide excess in addition to a possible common mechanism of control.[2]

5524

Electrocardiographic Study of Movements of the Heart with Change of Posture.

M. H. NATHANSON.

From the Department of Medicine, University of Minnesota, Medical Service, General Hospital.

The human electrocardiogram, as recorded, leading off from points on the surface of the body, is influenced by extracardiac factors, particularly by the effects of the tissues about the heart and the relation of the heart to the surrounding tissues. It is possible for a variation in the electrocardiogram to occur by a change in the extracardiac relations, even though the processes within the heart remain unaltered. Several observers have noted a variation in the form of the electrocardiogram on change of position of the body from the reclining to the lateral positions. There is no agreement as to the type and degree of the alterations nor as to the mechanism underlying these changes. The previous observations have been limited to a small group of subjects and no analysis of a sufficiently large number exists to justify definite conclusions.

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[2] Since this report has been in press our attention has been called to similar experiments in which sodium cyanide and sodium sulphide were injected into both common carotid arteries with denervation of the carotid sinus on one side. Heymans, Bouckaert and Dautrebande. *Archives Internationales de Pharmacodynamie et de Therapie,* 1931, **40**, 54.

In the present study, electrocardiograms were made of 60 individuals in 3 positions, in the reclining, in the left lateral and right lateral positions. The material consisted of normal subjects and of cardiac patients in whom a fixed heart was improbable, such as cases of hypertension heart and coronary disease. The latter were included to determine the effect of cardiac enlargement. The influence of such factors as habitus of the individual, size and displacement of the heart, was noted. Of the 60 cases, 55, or 91%, presented some definite electrocardiographic alteration on change of position of the body. In 5 cases the records were similar in the 3 positions. The change in the form of the electrocardiogram affected

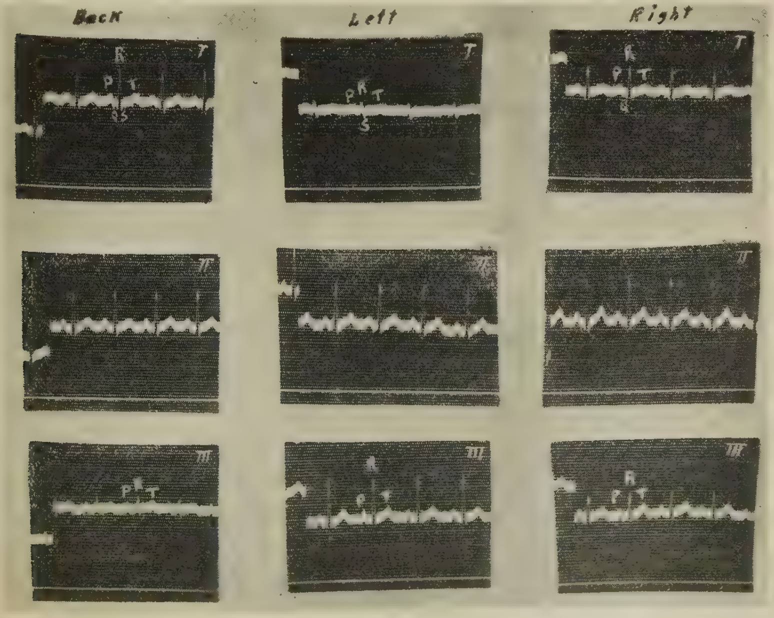

FIG. 1.
Leads 1, 2 and 3 of a normal individual in the 3 positions.

chiefly the QRS wave, although frequently the other waves were modified in the same direction but to a lesser degree. The most marked change was present in the first and third leads, the second lead usually being but slightly affected. These modifications in the amplitude and direction of the QRS can be expressed as variations in the degree of ventricular preponderance. Thus, following the usual terminology, any reduction of the R and increase of the S in lead 1, or increase in the R and decrease of S in lead 3 may be termed a change towards right preponderance. Any alteration of the waves in the opposite directions may be considered as a deviation toward left preponderance.

The most constant and marked changes occurred when the subject was turned from the reclining to the left lateral position. (Fig. 1.) With this procedure, the electrocardiogram altered in the direction of right preponderance in 83% of the cases, towards left preponderance in 6% and was unaffected in 11%. On shifting from the back to the right lateral position, the changes were less constant and usually less marked. In 30% there was no variation in the form of the electrocardiogram as compared with the record taken in the reclining position. Of the remaining 70% one-half changed in the direction of right preponderance and the other half deviated toward left preponderance.

In explanation of these electrocardiographic findings, the only

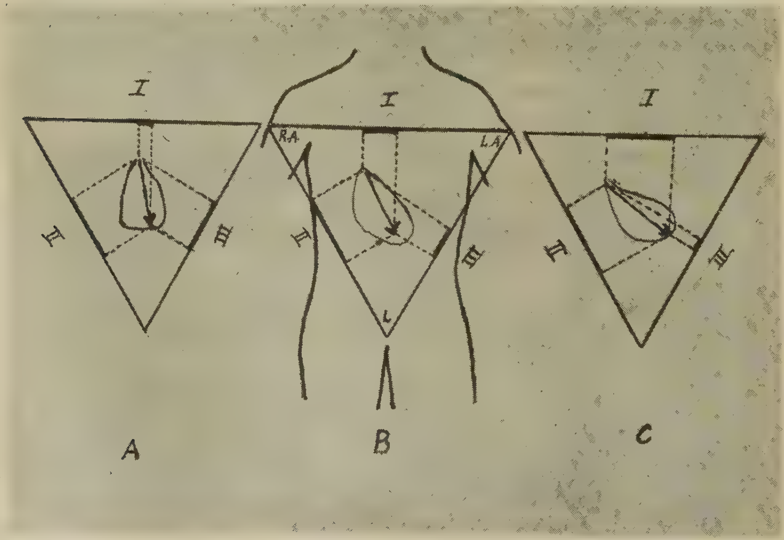

FIG. 2.
The schema of the equilateral triangle showing the effects of rotation of the heart about the anterior posterior axis.

extracardiac mechanism of sufficient influence to produce such changes is a shift in the position of the heart with the change in body position. Einthoven, Fahr and deWaart[1] have demonstrated that the effects of rotation of the heart as viewed in the frontal plane may satisfactorily be explained by the application of a schema in which the human body is represented by a plane in the form of an equilateral triangle. (Fig. 2, B.) The apices correspond to the right arm, R. A., left arm L. A. and both legs L, from which the current is led off to the galvanometer. The heart is in the center of the triangle and if a line drawn through it representing the direction of difference in potential, is projected on each side of the triangle, the proportional amount of current tapped by each lead is recorded. It is obvious from inspection of the triangles that rotation of the heart about the anterior-posterior axis will produce profound changes in the amplitude of the waves, especially in leads 1 and 3. (Fig. 2, A and C.) With the application of this method it is possible to interpret the changes in amplitude and direction of the QRS in leads 1 and 3 in terms of rotation of the heart. Thus, with the heart relatively fixed at the base and comparatively free at the apex, a change in the direction of left preponderance indicates a rotation of the heart about an anterior-posterior axis to the left (Fig. 2, C), while a change towards right preponderance indicates a rotation to the right. (Fig. 2, A.) Applying this to the findings noted in this report, it is clear that rotation of the heart about the anterior-posterior axis will not explain them, since the left lateral position resulted in a tendency to right preponderance in 83% of the cases. The possibility that upward displacement of the left diaphragm in the left lateral position could rotate the heart to the right sufficiently to account for the right preponderance needs little consideration. Roentgenologic studies of the chest in the 3 positions exclude such a possibility unless it be in very exceptional cases.

The effects of rotation of the heart about the longitudinal axis have been studied by 2 groups of investigators. Boden and Neukirch[2] found that rotation of the isolated perfused heart about the long axis to the left resulted in a tendency to right preponderance, while rotation to the right gave opposite effects. Meek and Wilson[3] arrived at the same conclusions in their studies upon the intact animal. Thus it is clear that the 2 possible types of rotation of the heart associated with a change from the reclining to the lateral positions have opposite electrical effects.

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[1] Einthoven, W., Fahr, G., und deWaart, A., *Arch. f. d. ges. Physiol.*, 1913, **150**, 275.

[2] Boden, E., und Neukirch, P., *Arch. f. d. ges. Physiol.*, 1918, **171**, 146.

[3] Meek, W. J., and Wilson, A., *Arch. Int. Med.*, 1925, **36**, 614.

With this in mind, the analysis of the findings in the present report indicates that on the change from the reclining to the left lateral position, the electrical effects of rotation about the longitudinal axis predominate in most instances, with occasional predominance of the effects of rotation about the anterior posterior axis. In approximately 10% the 2 opposite effects exactly neutralize one another as indicated by the absence of any alteration in the electrocardiogram. In the shift to the right lateral position there is approximately an even distribution of the effects, each type of rotation predominating in one-third of the cases and a neutralization of the effects in the remainder.

These studies suggest that some care must be observed as regards the position of the patient in the taking of electrocardiographic records. It is possible in many cases that a moderate rotation of the body, especially to the left, may sufficiently alter the record as to result in error, particularly in the interpretation of ventricular preponderance. These observations also indicate that an absence of variation in the electrocardiogram with change of posture is not invariable evidence of a fixation of the heart.

5525

A Rat Board for X-Ray, Photography, and Operative Procedure.

ALFRED T. SHOHL.

From the Babies and Childrens Hospital, Cleveland, and the Department of Pediatrics, School of Medicine, Western Reserve University.

The board for holding rats, shown in the accompanying illustration, will be found convenient for x-ray exposures, photographs or for an operating board. It was designed to obviate the danger to the workers of exposure to x-rays. We calculated that the number of exposures necessary was in excess of the dosage compatible with safety. With the present device, one person can do the work for which 3 were formerly required. The animal is placed in the holder outside the x-ray room and while the exposure is made, a second animal is made ready. Thus no worker is exposed to x-rays.

The apparatus shown was made from sheet aluminum. It can be made of steel or covered with lead as desired. The clips are battery clips with the teeth flattened to the plane of the clip and guarded with rubber tubing. The spring of the clip should not be strong enough to hurt the animal's paw. A test on the finger will determine whether the jaws should be bent or the spring altered. The clips

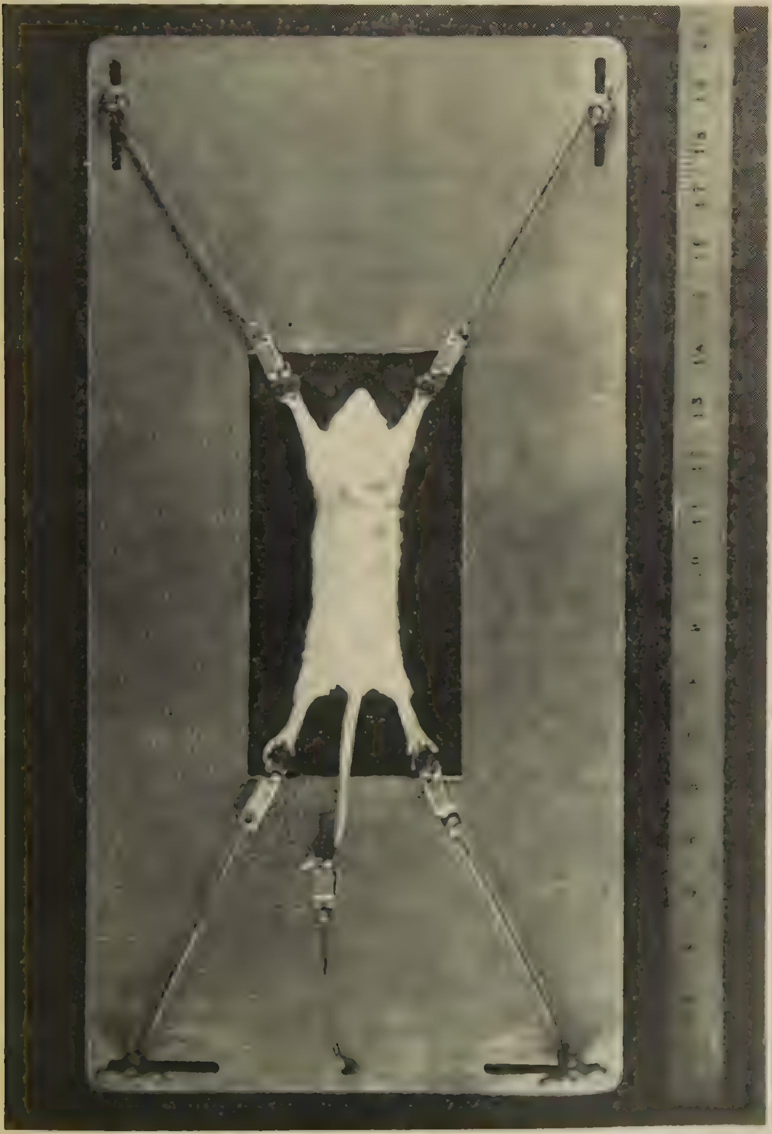

FIG. 1.

This photograph was made in the photographic studio of the Babies and Childrens Hospital. D. G. Shields, photographer.

are fastened to the frame with coiled spring wire. This permits the animal to be held firmly but is still elastic. Under such conditions the animals are restrained but not immobilized. They do not feel frightened and remain quiet and docile upon the board.

Mr. B. Dan, Supervisor of Equipment of the Institute of Pathology, Western Reserve University, has given suggestions for some mechanical features and his help is gratefully acknowledged.

5526

Pharmacology of Ruvettus Pretiosus, or "Castor-Oil Fish."

DAVID I. MACHT AND J. BARBA-GOSE.

From the Pharmacological Research Laboratory, Hynson, Westcott and Dunning, Inc., Baltimore, Maryland.

Ruvettus pretiosus is a deep-sea fish inhabiting the Southern Pacific, the flesh and bones of which contain a fat or oil known to South Sea Islanders as a purgative agent.[1] At the suggestion of Dr. E. W. Gudger, of the American Museum of Natural History, who described the biology of the fish,[2, 3] a pharmacological study was undertaken of the oil obtained through Mr. Donald G. Kennedy, of Vaitupu, Ellice Islands, South Seas. The physical and chemical constants of the specimen studied, as determined by Dr. Warren M. Cox, were as follows:

Density $\frac{25}{25}$	0.8725
Density $\frac{25}{4}$	0.8700
Iodine No.	74.42
Saponification No.	124.0
Refractive Index $\frac{25}{D}$	1.4628

Preliminary experiments revealed that 10 to 15 cc. of *Ruvettus* oil administered to a cat by stomach tube was followed by an abundant soft stool after 2 hours. In dogs, 25 to 30 cc. of the oil have the same effect. The oil being limited, a special method of pharmacological experimentation was devised for quantitative and comparative studies. This consisted in preparing a thick suspension of finely divided animal charcoal and introducing from 0.5 to 1 cc. by means of a "stomach tube" into rats previously fed on a standard dry diet and allowed to fast a day before the experiment. The rats are killed at the end of a suitable period of time, usually 50 minutes; and the entire gastrointestinal tract is excised and spread out on the table. The distance traversed by the carbon suspension, seen through wall, is carefully measured and expressed as a percentage of the total length of the intestine. 0.1 to 0.2 cc. of an oil or other substance to be examined is given in the same way together with the black emulsion; comparative figures are thus obtained as to the laxative effect of a substance and normal controls. *Ruvettus* oil is as laxative as castor oil but the pharmacodynamic mechanism is different. Castor oil produces purgation by virtue of its *saponifiable* fraction, namely, ricinoleic acid. When *Ruvettus* oil is separated into the saponifiable and unsaponifiable fractions, it is the *latter* which is purgative. Studies on isolated intestinal loops by Moreau's method show that the *Ruvettus* oil is not irritating and causes only slight increase of intestinal secretion and mucus and less congestion than is produced by castor oil. *Ruvettus* oil does not act as a purgative when injected subcutaneously or intramuscularly. Emulsions injected intravenously are not toxic, producing only transient fall in blood pressure in cats and dogs and not affecting the respiration at all. Further pharmacological study made a thorough chemical analysis of the oil imperative.

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[1] Lowe, *Trans. Zool. Soc., London*, 1841, **2**, 180.

[2] Gudger, *Boston Med. and Surg. J.*, 1925, **192**, 107.

[3] Gudger, *Am. Naturalist*, 1928, **62**, 467.

Warren M. Cox, Jr., made an exhaustive chemical study, while the pharmacological examination of numerous fractions and other compounds he prepared was carried out by the present authors. This work is still in progress but the findings so far obtained are here described.

The principal constituent of the saponifiable portion of *Ruvettus* oil was found to be oleic acid and the comparatively low iodine numbers obtained from various fractions studied indicated that there was very little unsaturation of a higher order than the mono-ethylenic. The more important *unsaponifiable* portion of *Ruvettus* oil was also fractionated by Dr. Cox; and its principal constituent is the *saturated* compound cetyl alcohol, $C_{16}H_{33}OH$. The most important *unsaturated* compound in the unsaponifiable portion of the oil is oleyl alcohol. Comparative pharmacological tests were made with ethyl esters of *oleic* and *hydroxy-oleic* acids, on the one hand, and with *cetyl acetate* and *oleyl acetate,* on the other. The following table shows the relative laxative effects for rats, by the above method, of olive oil, castor oil, *Ruvettus* oil and the principal fractions mentioned. In each case, 0.1 cc. of the compound was administered with a carbon suspension.

TABLE I.

		Average Distance Traversed (% of total length)
I	Controls	45
II	Olive oil	55
III	Castor oil	70
IV	*Ruvettus* oil	68
V	Saponifiable fraction	
	a. Saturated compound—ethyl oleate	63
	b. Unsaturated compound—ethyl hydroxy oleate	59
VI	Unsaponifiable fraction	
	a. Saturated compound—cetyl acetate	77
	b. Unsaturated compound—oleyl acetate	74

The most active compound from the purgative standpoint was *cetyl acetate.* The action of cetyl alcohols and their esters were studied. Eight hexadecanols were prepared by Dr. Cox and Professor Reid, and the 8 alcohols with their 8 acetates were obtained in small quantities but in pure form. Various hexadecanols and their acetates were examined by the rat method and also on isolated segments of cat's intestine by a method devised by the authors, to be described elsewhere. The various hexadecanols and their esters differ from each other pharmacodynamically both *quantitatively* and *qualitatively.* The 2 compounds which were strikingly stimulating to the intestinal movements are 2 ethyl-2 dodecyl-ethanol 1; 2 hexyl-2 octyl-ethanol 1.

A roentgenological investigation of all the compounds studied is in progress and results obtained so far completely agree with the findings described above.

5527

A Method for Interval Determinations of Liver Glycogen in the Same Dog.

I. Y. OLCH, F. E. WALTON AND W. S. SCRIVNER.
(Introduced by E. A. Graham.)

From the Department of Surgery, School of Medicine, Washington University.

In most experiments heretofore described for the study of changes in liver glycogen, a series of animals are used, one of which is sacrificed at the end of a given period. Cori[1] has described a method of obtaining several samples of liver from the same animal for the determination of liver glycogen. The advantage of repeated observations in the same animal is that the individual variations in liver glycogen between animals of the same species are avoided. In normal dogs, kept and fed under uniform conditions, we have found this to be from 1.3% to 7.2%. Varela, Duomarco and Munilla[2] found it to be from 0.74% to 4.68%. Similar differences are found in other animals.

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[1] Cori, C. F., Cori, G. T., and Pucher, G. W., *J. Pharm. and Exp. Therap.*, 1923, **21**, 377.

To carry out repeated determinations of liver glycogen at stated intervals on the same animal, it seemed necessary first to bring a part of the liver to the body surface, where pieces could be excised. This is done as follows: Intra-tracheal anesthesia is given with the Erlanger positive pressure apparatus and through an incision on the right side, parallel and about 5 cm. anterior to the spine, the 4 lower ribs are then removed. The pleura and diaphragm are next opened in the same axis. The corresponding cut edges of diaphragm and pleura are sutured to each other, thus closing the pleural cavity. The thin attachments of the liver are then cut, allowing mobilization of the 2 lateral lobes or more. Any other procedure on other abdominal organs can be carried out at the same time, through the same incision, such as ligation of the bile ducts, removal of the pancreas, production of intestinal obstruction, etc. The liver is brought to the surface more by pushing in the collapsed chest wall than by traction. The skin and the subcutaneous tissues are closed with just enough sutures to keep the exteriorized portion of liver from slipping back. About 100 gm. of liver can thus be brought to the surface. This is dressed with vaseline gauze and a dry pad, which are changed daily.

In our experiments, small pieces of liver weighing about 1.5 to 2 gm., are excised from the exposed liver and immersed immediately in about 2 cc. of 60% KOH, which is contained in a 50 cc. Pyrex graduated centrifuge tube, all of which have been previously weighed. All are weighed again to determine the exact weight of the liver sample. From here on the method is that of Pfluger and the sugar is determined by the Shaffer-Hartman method. The mass of liver which is exteriorized is covered with visceral peritoneum and inasmuch as the latter is free of pain fibers, the liver can be handled and pieces removed without any sensation of pain or discomfort to the animal. This makes it possible to omit an anesthetic and so avoid the effect of the latter on carbohydrate metabolism. The bleeding from the cut surface is easily controlled with pressure. In this manner a complete experiment can be run on one animal, the control value of liver glycogen being that found in the liver sample before the administration of the glucose, adrenalin, or insulin.

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[2] Varela, B., Duomarco, J., and Munilla, A., *Z. f. d. ges. Exp. Med.*, 1930, **72**, 457.

5528

Studies of Renal Metabolism.

C. P. RHOADS, D. D. VAN SLYKE, ALMA HILLER AND A. ALVING.

From the Hospital of the Rockefeller Institute for Medical Research.

A kidney is drawn out of the body cavity of a dog and sutured under the skin of the back, with the renal vein located in such a position that it can be punctured through the skin with a needle. The metabolism of the kidney has been studied by simultaneous analyses of arterial and renal bloods and of urine secreted during the observation periods. The rate of blood flow through the kidney has been estimated by comparing the amount of urea removed from a unit volume of blood in passing through the kidney with the amount excreted per minute. In most of our experiments only one kidney at a time has been studied; one is brought out under the skin, and the other is removed, in order to simplify the experiments. In a few controls in which one kidney was brought out and the other left *in situ* excretion was similar in both.

Through a single kidney in dogs of 15 to 20 kilos weight the blood flow is in the neighborhood of 200-250 cc. per minute. Increasing the urea content of the blood as much as 10-fold does not significantly accelerate the blood flow. The urea excretion increases 10-fold, but the increase is due to the fact that the proportion of blood urea removed during perfusion of the kidney remains constant, so that the amount removed per liter of blood passing through the kidneys rises in proportion to the amount present in the blood. This explains the manner in which the blood urea clearance is kept constant during wide fluctuations in blood urea content.

The proportion of oxygen removed from the blood by the kidney is rather low, 10 to 20% of the arterial oxygen content as a rule. Increasing the work of the kidney in the form of urea excretion does not markedly affect the oxygen consumption.

Occasionally we have noted temporary cessation of removal of urea from the renal blood. Such cessation apparently occurs as the result of trauma in tapping the renal vein. The cessation appears to be an all or none phenomenon. When it occurs the urea content of the renal vein rises as high as, and sometimes higher than, the urea content of the arterial blood.

The occurrence of renal blood urea contents markedly higher at times than arterial urea contents appears to be proof of the occurrence of reabsorption of urea from the kidneys.

5529

Production of Anatomical Lesions in the Isolated Organ.

JEAN OLIVER.

From the Department of Pathology of the Long Island College of Medicine, Brooklyn, N. Y.

Since the frog's kidney has proved such a suitable organ for the investigations of the physiologist it seems likely that an examination of its structure and activity under abnormal conditions might be of value. The present report concerns one aspect of such a study. We have previously described the lesions in the frog's kidney when renal toxic agents, including potassium bichromate, corrosive sublimate, uranium nitrate and snake venom, are injected into the living animal.[1] The next step has been the elaboration of a method to test their functional activity, and in this procedure it was found advisable to isolate the organ and perfuse it with a modified Locke's solution.[2] While it was functioning normally under these controlled conditions the same toxic agents were administered by way of the perfusion fluid and the resulting disturbance of functional activity noted. These will be described at another time.[3] The tissues were then fixed and examined histologically.

In this study our first procedure was to determine that normal perfusion of the organ produced no structural changes. It was found that after 6 hours of such treatment the most delicate cytological structures such as the brush border of the epithelial cells, the mitochondrial elements and the achromatic spindle of mitotic figures, which are occasionally found in the frog's kidney, were entirely normal in appearance.

In the tissues of the damaged kidneys all the pathological lesions that had developed in the kidneys of the living animal after poisoning with the same toxic agents were observed, except those which require a longer time for their development than was available in the experiment or which depend on the presence of the circulating blood. The epithelial lesions were localized to that part of the tubule which was most susceptible in the living animal. They consisted of necrosis with the typical nuclear changes of pyknosis and karyolysis, desquamation and cast formation. The granular changes involving the mitochondrial apparatus, cloudy swelling, agglutination and clumping or solution of the granular structures were especially well reproduced. In the glomeruli, the lesions were modified from the appearance seen in the lesion in the living animal by the absence of blood in their capillaries, but the same deposits of fibrinoid material were present in Bowman's space, and similar areas of necrosis were evident in their tufts. In the interstitial tissues of the organ, areas of edema identical to those found in the kidneys of the poisoned animals were seen.

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[1] Oliver, J. and Smith, P., *J. Exp. Med.*, 1930, **52**, 181.

[2] Oliver, J. and Shevky, E., *J. Exp. Med.*, 1929, **50**, 15; *Am. J. Physiol.*, 1930, **93**, 363; MacKay, E., and Oliver, J., *J. Exp. Med.*, 1930, **51**, 161.

[3] *J. Exp. Med.*, in press.

These findings can be summarized by the statement that an experimental nephritis was produced extra-vitally, if we may use such a term, with all the essential lesions that develop *in vivo*. The structural changes are obviously not the result of mechanical effects due to variations in such physical forces as the osmotic pressure of the artificial perfusion fluid, for these kidneys were functioning in an entirely normal manner before the lesion was produced, concentrating urea or dyes many times, retaining sugar or diluting salts, and as we shall show later, the disturbances of functional activity were similar to those which follow the action of the same toxic agents on the kidney of the living frog. The lesions can therefore only be explained as the result of pathological processes which were apparently similar to those which occur *in vivo,* since their structural and functional effects were similar.

The method of Ludwig may, therefore, also serve as a means of study of anatomical as well as functional processes. The value of a single approach towards these two aspects of vital activity is obvious. The possibility of control that the method makes possible is analogous to that obtained by tissue culture, but with it not only tissue reactions may be examined, but the reactions of an entire organ to controlled conditions may be followed both structurally and functionally.